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EFFECT OF EPINEPHRINE, CORTISONE ACETATE AND ADRENOCORTICOTROPIN ON CIRCULATING EOSINOPHILS, PLATELETS AND MARROW MEGAKARYOCYTES OF INTACT AND SPLENECTOMIZED ALBINO RATS

by

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I. INTRODUCTION

Accumulating evidence indicates convincingly that certain hormones exert a regulatory influence on certain phases of blood cell formation and destruction (Crafts, 1941; Dameshek, 1946; Doughaday, Williams and Daland, 1948; and Sims et al., 1951). The endocrine glands that seem to be implicated include the pituitary, thyroid, gonads and the adrenals. In addition, the spleen seems to be involved in the production and delivery of blood platelets from the bone marrow (Dameshek and Miller, 1946). In a series of experiments upon guinea pigs, Ungar (1945) concluded that the removal of the spleen inhibited the pituitary-adrenal response to stress in these animals. He interpreted this reaction as evidence for the hormonal activity of the spleen. As yet, Ungar's work has not been confirmed.

At the present time considerable attention has been focused on the effects produced by adrenocortical factors upon the white blood cell elements of the blood. A decrease in the eosinophil level in the circulating blood of the animal under stress is recognized as a sensitive indicator of the presence of increased quantities of adrenocortical and adrenocorticotropic factors in the plasma (Thorn et al., 1953). However, no study seems to have been done in which the eosinophil counts were made concurrently with those of other blood elements, in animals under hormonal stress, as a measure of the adrenal activity the animals were undergoing at that time. Although some data have appeared on the manner whereby

cortical hormones influence granulocytes and erythrocytes in the peripheral blood, only fragmentary and conflicting information is available as to the effect upon the number of circulating blood platelets and upon their formation in the bone marrow.

Wallace (1951) recently described, in human beings, a new clinical and pathological entity, named thrombotic thrombocytopenic purpura. The principal symptoms of this syndrome are widely disseminated platelet thrombi throughout the small arterioles, venules and capillaries; joint pains; a purpuric rash; hemolytic anemia; and thrombopenia. Although the etiology of this disease is completely unknown, it is plain, however, that blood platelets are directly involved. Obscure relationships of this type indicate the value of additional attention to the role of the blood platelet in mammalian physiology. Of additional interest is the fact that the symptoms of thrombotic thrombocytopenic purpura include joint pains. Because of this, Wallace (1951) suggests that the newly described entity may be related to the diseases of the diffuse collagen group. Some of the latter have responded to treatment with the adrenocortical hormones. In view of such interrelationships among platelets, eosinophils, marrow megakaryocytes, and adrenal activity, experimental procedures were initiated to investigate the possible effects of epinephrine, cortisone and adrenocorticotropic hormone* on the number of circulating blood platelets and marrow megakaryocytes. The activity of the adrenal glands and/or the plasma concentration of the adrenal hormones, in each case, was measured, concurrently, by eosinophil counts.

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* Hereafter referred to as ACTH.

II. REVIEW OF LITERATURE

A voluminous literature concerned with the study of blood platelets, eosinophils and marrow megakaryocytes has accumulated since the turn of the twentieth century. Recently, even more attention has been devoted to these protoplasmic entities in connection with investigations related to physical and physiological stresses. Many of these older and newer references do not come within the scope of the present review; only pertinent historical and recent papers are cited. However, if available, comprehensive surveys within certain areas are listed when their content is of such nature as to warrant their inclusion as background literature.

A. The Cellular Elements

1. Blood platelets

A survey of the pertinent literature concerning the morphology and physiology of blood platelets, from their original description in 1842 by Donne to 1950, was prepared by Ottis in 1951. Since 1950 considerable work has been done on the morphology of platelets by Fonio (1951) with the electron microscope. A method by which platelets could be collected in a resin container as a by product of routine blood collection was described by Freeman (1951). The platelets collected by Freeman's method were not damaged as they were by the usual method of centrifugation and washing. Hirsch and Gardner (1952) investigated the viability and survival time *in vitro* and *in vivo* of platelets isolated and concentrated by various methods.

Since 1950, Kissmeyer-Nielsen (1951a, 1951b, 1951c) made a number of investigations of bone marrow and blood thrombopoiesis in various thrombocytopenic conditions such as pernicious anemia, acute and chronic thrombocytopenic purpura and thrombocytopenias which are secondary to various splenomegalies. He concluded that the mechanism causing thrombocytopenia in all these conditions are similar; namely, that the decreased number of platelets was due not to a decreased number or a "shift to the left" of the megakaryocytes in the marrow, but to abnormal function manifested as diminished platelet production. The basic cause remains unexplained although the above author favors alteration in functioning of the spleen, or perhaps of the entire reticular-endothelial system.

Much evidence has been accumulated to show that transfused platelets have a short survival time of less than one hour up to only three hours in thrombocytopenic purpura (Hirsch and Gardner, 1952; and Stefanini et al., 1952). In a search for a mechanism to explain the low platelet levels in these hemorrhagic diseases, Stefanini and coworkers (1952) were unable to demonstrate any evidence for selective sequestration of the platelets by the spleen. More recently, Stefanini, Chatterjea and Dameshek (1952) reported negative evidence on platelet removal in pulmonary circulation.

2. Eosinophils

Since the middle of the nineteenth century, when Jones (1846) first described the eosinophil, and, later, when Ehrlich (1879) first stained its granules red, the eosinophil has remained a comparatively mysterious cell. The revived interest of clinicians in the eosinophil is the result of two circumstances: first, the discovery that adrenocortical activity produced

eosinopenia; and second, the recognition that absolute eosinophil counts were easy to make and were more accurate than differential counting of eosinophils from a stained blood smear (Best and Sampter, 1951).

The first publication to state that adrenocortical activity was related to eosinopenia was that of Hills, Forsham and Finch (1948). They found decreases in the number of circulating leucocytes, including eosinophils, after treating patients with ACTH. Investigations which followed indicated that numerous stress stimuli caused eosinopenia through activation of the pituitary-adrenal axis. Included among examples of such stress agents are surgical operations (Laragh and Almy, 1948; Roche, Thorn and Hills, 1950), coronary occlusions (Gabrilove, 1950), electroshock (Altschule, Parkhurst and Tillotson, 1949) and epinephrine (Drury, 1950).

There is a question as to whether the disappearance of blood eosinophils following increased hormonal output is due to decreased production, sequestration in the blood vessels of large organs, increased destruction, or the movement of the eosinophils into the tissue beds. There does not appear to be a decrease in eosinophils in the bone marrow at the time of maximum eosinopenia (Rosenthal et al., 1950), although an increase in immature eosinophils was noted by Durgin and Meyer (1951). The evidence for sequestration is conflicting. Although Spain and Thalhimer (1951) found that eosinophils accumulate in the spleen of mice during the period of eosinopenia, other workers such as Solomon and Humphreys (1951) were unable to demonstrate eosinophil sequestration in the rat spleen.

The suggestion has been made that eosinopenia may result from movement of cells into the extravascular loose connective tissue (Dougherty, 1950). Pycnosis and fragmentation of blood eosinophils have been

observed by Padawer and Gordon (1952) and by Poel and Becken (1952) during eosinopenia. Padawer and Gordon (1952) also observed that as the circulating eosinophils decreased in number, the eosinophils of the peritoneal fluids increased. A study of the comparative action of epinephrine and ACTH in the production of eosinopenia in mice was made by Speirs and Sullivan (1953). These workers concluded that epinephrine must act directly upon the adrenal cortex in some manner.

In general, it can be concluded that eosinopenia may be due not only to the release of adrenal corticoids but also to some other reaction. However, since the presence of adequate amounts of 11-oxycorticoids always produce eosinopenia, (Denison and Zarrow, 1954) this reaction may be used to determine the presence or absence of these hormones.

3. Bone marrow megakaryocytes

Howell, in 1890, discovered these giant cells in bone marrow and gave them the name of megakaryocytes to distinguish them from the osteoclasts. Wright (1906) established the megakaryocytes as the progenitors of the blood platelets. Later, Bunting (1909), Downey (1913), and Minot (1922) confirmed Wright's work. Also, clinical evidence to substantiate Wright's theory of platelet origin has been contributed by Duke (1915), Gunn (1931), and Dameshek and Miller (1946). Histochemical studies by Wislocki, Bunting and Dempsey (1947) disclosed that the protoplasm of the blood platelet reacted to various histochemical tests in the same manner as the cytoplasm of the megakaryocyte. Kerhulas, Ohler, Warren and Belko (1951) established criteria for recognizing the various developing forms of the megakaryocyte. Smith and Butcher (1952) have compiled a detailed review

on the incidence and significance of megakaryocytes in normal and diseased tissues.

B. Adrenal and Adrenocorticotropic Effects on Platelets

1. Epinephrine

The original suggestion that epinephrine might have an effect on the blood platelets came from Cannon and Gray (1914). These authors found a shortened coagulation time in experimental animals after epinephrine had been injected. Edmunds and Nelson (1925) demonstrated a general increase in circulating blood cells after epinephrine and thought that this was due to marrow stimulation, although no marrow studies were actually done to substantiate this concept. Lucia, Aggeler, Husser and Leonard (1937) observed an increase in both red and white blood cells after injections of epinephrine. Hungerford (1949) studied the circulating leucocytes and found that they decreased in number in the rat following epinephrine. White, Ling and Klein (1950) emphasized that the effects of epinephrine and ACTH upon leucocyte counts were similar. Gemzell (1952) observed that after epinephrine the increased plasma concentration of ACTH in the rat was not followed by an increased rate of synthesis of ACTH by the anterior pituitary. Continho et al. (1953) contended that epinephrine is a non-specific stressor rather than a pituitary-adrenal "trigger". Leslie, Blahd and Adams (1953) found that epinephrine was a pituitary-adrenal "trigger", but that its activity was very brief.

2. Cortisone and ACTH

a. Historical. In March of 1936, Kendall, Mason, Meyers and Allers announced that nine separate, but closely related, compounds had been

extracted from the adrenal cortex. One of them, Compound E (C_{21} H_{30} O_5), was called cortisone. Mason, Meyers and Kendall (April, 1936) revised the formula of Compound E to C_{21} H_{24} O_3 and described it as a crystalline hormonal steroid. In the same year, Reichstein (1936) also described a compound very similar to Kendall's Compound E. Later, Kendall (1941) perfected methods for the assay of the various adrenocortical hormones. Hench, Kendall, Slocumb and Polley (1949) were the first to use cortisone clinically on human rheumatoid arthritic patients.

It was demonstrated by Smith in 1927 and later amplified by him in 1930 that atrophic adrenals of the hypophysectomized rat could be restored to their normal histology and to normal or near-normal weight by homo-transplants of pituitary gland. Collip, in June 1933, announced that the thyrotropic fraction of anterior pituitary extracts gave evidence of containing an adrenocortical stimulating principle. In August of the same year, Collip et al. presented conclusive proof that an adrenotropic factor was present in the anterior lobe of the pituitary gland. Moon (1937) achieved a satisfactory assay method for the adrenotropic principle, using rats as the assay animal. Thorn et al. (1947) were the first to demonstrate the action of ACTH clinically. Their laboratory observations showed that the hormone stimulated the adrenal cortex.

b. Platelet effect. The literature on the effect of cortisone and ACTH upon the circulating blood platelets is contradictory and, therefore, confusing. This may be due partly to the questionable purity of the extracts used in the earlier trials as well as to improper dosages. Zondek and Kaatz (1936) found a decrease in the number of blood platelets with cortical extracts, while Adams (1949) observed no significant effect

upon platelets or megakaryocytes after cortisone or ACTH injections into rats. Monto, Brennan, Margulis and Smith (1950) demonstrated no change in number of platelets after ACTH administration, but, in contrast, Wintrobe (1950) noted a temporary platelet increase in thrombocytopenic patients after administration of ACTH. Jacobson and Sohier (1952) found increases in platelets in normal human beings after ACTH injections, whereas, Lauridson, Beldo and Warren (1952) claimed a very significant decrease in platelet numbers in patients treated with ACTH. The blood platelet count was not altered in a study made by Stickney and Mills (1952) after ACTH was administered to children.

C. The Spleen

From being considered as of no real value to the organism, the spleen has come to occupy a rather insecure position as a gland of internal secretion (Perla, 1936). A wide variety of functions has been assigned to the spleen from time to time, most of them involving the life cycle of the blood corpuscles. It has been stated that the spleen manufactures red blood cells; and, on the other hand, it has been claimed that red blood cells are destroyed in this organ. In the same way, the spleen has been regarded by some as a place where white blood cells are produced and by others as a place where they are destroyed. Evidence of an endocrine function of the spleen is still meager and to some extent conflicting.

The spleen was known to the ancients; the Greeks considered it as essential to life. Aristotle referred to it and Erasistratus stated that it was useless. It was regarded by Greeks and Romans as detrimental to a runner, an idea that persisted to the time of Shakespeare. It is

frequently stated that ancients removed the spleen of runners to increase their speed. These historical data were reviewed by Krumbhaar (1926) in his paper on the functions of the spleen.

According to a review by Downs (1948), the first carefully planned experiments directed toward elucidation of the function of the spleen were carried out by Bardelaben in 1841. The results of the complete removal of the organ were, briefly: a transitory decrease in the number of red, and an increase in the number of white corpuscles in the circulating blood; an increased activity of the bone marrow and lymphatic glands; no apparent ill effect on life. These experiments are particularly noteworthy because of the care with which they were planned, the thoroughness with which they were carried out and the conclusions drawn.

A relationship of the spleen to hematopoiesis was recognized as a result of Bardelaben's experiments, which were confirmed by other investigators such as Downs (1948). The unsuccessful outcome of the extirpation of the spleen in leucocythemia led to a turning of attention to the anemias. At the beginning of the twentieth century any syndrome of splenic enlargement and anemia was classified as splenic anemia. If the spleen was responsible for the destruction of red blood cells, then it seemed to follow that a condition such as pernicious anemia should be improved by removal of the organ. Consequently, during the second decade of the century splenectomy was resorted to in a number of cases. The results were inconclusive.

During this time, Whipple, Hooper, and Robscheit had been doing their fundamental works on the anemias. Their report, in 1920, on the influence of meat, liver, and various extractives on blood regeneration

in dogs with simple anemia, produced by bleeding, is the foundation on which was developed much of the present knowledge of blood cell formation. From this study came the use of liver in pernicious anemia. Minot and Murphy (1926) found that feeding a half pound of liver daily brought about improvements in patients with pernicious anemia. It later became clear that the fraction of liver which was curative in pernicious anemia was not the one which was effective in the anemia of bled animals. These discoveries led to the abandonment of the surgical procedure of splenectomy as a mode of treatment of anemia.

With the rising interest in internal secretions, the spleen was not neglected, although it did not receive as much widespread attention as some of the recognized endocrine glands. During this period the most productive group of workers was that headed by Pearce, with Musser and Krumbharr and a large number of associates. Among others who were interested in trying to establish definite knowledge concerning the way the spleen functions were Leake, Holloway and Blackford, Eddy and Downs, Danilewsky, Stradomsky, and Mouzon.

Investigation of the part the spleen plays in the formation and destruction of blood elements has followed, mainly, four lines: microscopic examination of the spleen; counting the cells in the blood entering and leaving the organ; the results of splenectomy; and the effects of administration of splenic substance or extract.

The development of the concept of the spleen as a gland of internal secretion has taken place gradually during the past quarter century. In 1915, Frank made accurate studies of "essential thrombopenia" and postulated a marked diminuation in platelet production by the megakaryocytes.

Frank (1915) is incorrectly quoted by most observers as having suggested a splenic effect upon megakaryocyte-platelet growth. In the following year, Kaznelson (1916) suggested splenectomy as a therapeutic maneuver in a chronic case of thrombocytopenic purpura. The results of the first operation were brilliant, but in the next two cases only temporary increases in platelets resulted (Kaznelson, 1919). Since that time, the favorable effect of splenectomy in thrombocytopenic purpura has been amply confirmed (Dameshek and Miller, 1946). The quick recovery, after splenectomy, of many desperately ill patients, bleeding spontaneously from all orifices, is one of the most dramatic events of medicine and must immediately implicate the spleen as of prime importance in the disease. In confirmation of this, the injection of splenic extracts from patients with the disease has, in the hands of several investigators, resulted in the development of thrombocytopenia in experimental animals (Troland and Lee, 1938; Hobson and Wills, 1940; Rose and Boyer, 1941; and Cronkite, 1944). In a study on the platelet and megakaryocyte values, before and after splenectomy, in patients with thrombocytopenic purpura, Dameshek and Miller (1946) concluded that the low pre-splenectomy platelet-megakaryocyte counts and the high post-splenectomy platelet-megakaryocyte values indicated that an inhibitory effect of the spleen upon the bone marrow was present and active in thrombocytopenic purpura. In the same study Dameshek and Miller (1946) ruled out, by some very thorough histological studies of splenic biopsy material, the possibility of phagocytosis of platelets by the spleen as a significant factor.

Ungar reported in 1945 that splenectomy protected his animals against pituitary-adrenal response in a series of clotting time tests.

Ungar's description of materials and methods were inadequate, however, and it has been impossible to repeat his work. The data of Drury (1950) showed that the spleen is involved in neutrophilia and lymphopenia which follows three hours after the injection of adrenaline into white rats. Work by Johansen in 1953 seems to indicate that the human spleen can absorb a factor that normally promotes the function of the bone marrow.

In completing this review of the spleen, it should be noted that several present day texts of physiology still ascribe platelet increases following epinephrine to splenic contraction (Best and Taylor, 1945; Evans, 1952; Zoethout and Tuttle, 1952). This view is contrary to present-day evidence from critical experiments.

III. MATERIALS

The 158 white rats utilized in this investigation were young adults of the Rolfmeyer-Holtzmann strain, aged 2-3 months, weighing between 200-300 grams; approximately equal numbers of males and females were used in each experiment. The animals were maintained on Purina Laboratory Chow, supplemented by chopped carrots and pork liver once a week. Clean drinking water was available at all times. This diet proved adequate for normal growth and normal stability of body weight throughout the period of the investigation. The animals were housed in clean, well-ventilated metal cages; the sexes were kept separate. Clean wood shavings, changed weekly, were used for bedding.

Rats were always ordered to arrive about 30 days before a specific experiment was to be started. This period gave the animals an opportunity to adapt to changes in handling, diet and general maintainance. During the latter part of the acclimation, basal peripheral blood and bone marrow counts were made on each rat.

Any animal that showed the least sign of an existing pathology was withdrawn from the investigation and destroyed. A pilot experiment, previous to the reported investigation, ascertained that the strain was free of Bartonella muris. In rats infected with this disease the removal of the spleen is fatal. A macrocytic, hyperchronic anemia develops, followed by death of the animal (Weinman, 1938).

The cortisone acetate used in this investigation was procured

through the courtesy of Dr. E. Alpert of Merck and Company. Parke, Davis and Company epinephrine (1:1000) was used in the epinephrine experiment, and Armour High Purity Acthar Gel was used in the ACTH experiment.

IV. METHODS

A. Blood Platelets

The method for blood platelet determinations was described in detail by Ottis (1951). After experimenting with various platelet diluents, it was found that Rees and Ecker fluid* (1923), with its equivalent isotonic sodium citrate component as an anti-coagulant, formalin for fixation, and brilliant cresyl blue for staining, was the most satisfactory fluid. The solution was made up fresh each week and kept under refrigeration. Before using, the fluid was always filtered, and the amount left over at the end of the day was discarded. Spencer Bright-Line hemocytometers were used for platelet counts throughout this study. These hemocytometers were not used for any other purpose. Exacting cleanliness was practiced in maintaining and storing the hemocytometers and cover glasses. Hellige Zero-Error red blood cell pipettes were used to dilute the blood sample for the platelet counts. The maximum error for these pipettes, at all intervals, was less than 1 per cent, as compared to the $\pm$ 5 per cent tolerance allowed by the National Bureau of Standards. These pipettes were siliconized after the method described by Ottis and Tauber (1952) to prevent fragmentation and clumping of the blood platelets in the sample.

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* Formula for Rees and Ecker fluid

Sodium citrate	3.8 gm.
Formaldehyde	0.2 ml.
Brilliant cresyl blue	0.1 gm.
Distilled water	100.0 ml.

To obtain the blood sample for platelet determinations, the rat was lightly etherized, placed on the operating table and its tail cleansed with soap and water, followed by 70 per cent alcohol. The tip of the tail was then snipped, and free-flowing blood was drawn off into the red cell pipette to mark 0.5; the pipette tip was wiped off, and the dilution with Rees and Ecker fluid completed to mark 101. To decrease the possibility that the platelets might undergo fragmentation, the pipette was always quickly rinsed with the diluting fluid before drawing the blood to the 0.5 mark. The filled pipette was then shaken for exactly 2 minutes; three drops were expelled, and both chambers of one hemocytometer block loaded. The platelets were then allowed to settle for exactly ten minutes, after which platelets in the 5 "red squares" were counted in each chamber of the hemocytometer. The total number of platelets in a chamber was multiplied by 10,000 to find the total number of circulating platelets per cubic millimeter (Aggeler, Howard and Lucia, 1946). However, if the number of platelets from the two chambers deviated more than 8 per cent, the count was discarded and a new sample was taken. If the desired percentage check was obtained, an average of the two chambers was taken for the number of platelets per cu. mm. for that animal.

A superior optical system was necessary for platelet enumerations. In this investigation a research type binocular microscope equipped with a 44x objective and 10x oculars was used. A research type of microscope lamp was used, as the quality of illumination was very important.

After the count was tabulated, hemocytometers and pipettes were cleaned with Haemo-Sol solution, distilled water, alcohol, and ether, in that order. Pipettes with cracked or broken tips were immediately discarded.

B. Eosinophils

The greatest handicap in working with eosinophils was the relatively small number of these leucocytes found in normal blood. When using the blood film method, large numbers of white cells must be counted in order to determine accurately the percentage of eosinophils present. However, this difficulty has been overcome to some degree in clinical work by Randolph (1944) and Thorn et al. (1948) who have reported, for human blood, diluents that stain the eosinophils and destroy the other blood cells. Such diluents are based on the known fact that the eosinophil is the most resistant cell of the blood, especially to many hypotonic and acetone solutions. Randolph (1944) used a propylene glycol diluent, and Thorn et al. (1948) used an eosin-acetone diluting fluid for direct counting of eosinophils.

In a preliminary investigation, differential counts were made from blood films prepared in the conventional manner and stained with Wright's stain. However, for the purposes of the present investigations, this method was found too time-consuming for the large number of rats which were used. A search of the literature did not uncover any specific eosinophil diluent for use on blood other than that for human beings, although investigators such as Cunningham and Tompkins (1938) have used a number of vital dyes to study the blood picture as a whole in some experimental animals. Therefore, for the present investigation, it was advisable to work out a diluent which could be used on the eosinophils of rodents. An experimental study was undertaken to determine whether it was possible to devise a diluting solution which would destroy the red

blood cells and all the white cells except the eosinophils in rat blood. Numerous water and mild alkaline solutions did this, but the fragility of the eosinophil was so increased that any shaking of the diluting pipette caused them to disintegrate rapidly. Acetone was found to be more effective in preventing the breakdown than any other substance used. Propylene glycol, as recommended by Randolph (1944) for human blood, was tried but was found to delay the settling time to over 30 minutes. Acetone in very dilute solutions (less than 5 per cent in distilled water) caused lysis of all of the rat blood cells, especially after shaking. As the concentration of acetone was increased to 25 per cent, more and more eosinophils remained intact. A 22 per cent solution was found to give the best results with rat blood. At this concentration the eosinophils remained intact with distinct cell membranes, while the other granulocytes formed hazy images or "ghosts" in the background. The red blood cells were completely hemolysed. Either pholoxine-B or eosin was a good stain in the acetone medium for bringing out the eosinophilic granules. A small amount of detergent was added to obtain a better distribution of cells in the hemocytometer chamber. The following formula was found most satisfactory for rat blood and was used throughout the present investigation:

1.0% Eosin solution ..... 5 ml.
0.5% Syntax solution ..... 1 ml.
Distilled water ..... 33 ml.

Just before using, add
Acetone, C. P. ..... 11 ml.

It was found better to prepare the diluent each time it was used. Stock solutions were kept on hand and could be mixed quickly.

The rat eosinophil was recognized because of the following

characteristics. Granules within the cell stand out as distinct red bodies and are arranged as a mass in the center of the cell, surrounded by an annular nucleus. Usually the cell outlines were seen only faintly, and the nucleus was nearly colorless. Ordinarily, recognition of the eosinophil was accomplished with the lower power (100x) of the microscope. If identification of a cell was in question it was necessary to switch to the high power (440x) for a positive identification.

The diluting pipettes used for eosinophil counts were Hellige Zero-Error white blood cell pipettes with a maximum error of less than 1 per cent, at all intervals, as compared to the National Bureau of Standards tolerance of $\pm$ 3 per cent. Special larger Fuchs-Rosenthal hemocytometers were available for eosinophil counts. This type of hemocytometer has a depth of 0.2 mm. with a ruled area of 4 x 4 mm.; in other words, a volume of 0.2 x 4.0 x 4.0 or 3.2 cu. mm. The larger chamber made it possible to observe and count more eosinophils per chamber and thus resulted in an increase in accuracy of the absolute eosinophil counts. The rat eosinophils were always examined under bright light, using a blue glass daylight filter.

To obtain blood samples for eosinophil counts, the same procedure, as previously outlined for blood platelet determinations, was followed. The two samples were taken concurrently, with the eosinophil pipette being filled first. Free-flowing blood was drawn to 0.5; and the diluting fluid, to 11.0, giving a dilution of 1:20. The eosinophil pipette was shaken first for 25 seconds, three drops expelled, and the two chambers of the Fuchs-Rosenthal hemocytometer loaded. Both chambers were then counted immediately, and the total of both chambers multiplied by 3.12 (Best and

Sampter, 1951) for the absolute number of eosinophils per cu. mm. of rat blood.

Experience demonstrated that the direct method described above is quicker, easier, and more reliable than the indirect method using stained smears. This agrees with the published reports of others, such as Randolph (1944), Discomb (1946), and Speirs and Meyer (1949), who have used modifications of the direct technique.

C. Bone Marrow Megakaryocytes

Two methods for the study of femoral marrow of the rat have been perfected, one by Mayer and Ruzicka (1945), and the other by Crafts (1946). These methods did not meet the needs of the present experiment, however, because they entailed the sacrifice of the experimental animal before the marrow sample could be obtained. A method comparable to the sternal marrow biopsy used in clinics on human patients was needed. Iliac marrow aspiration had been worked out by Sawitsky and Meyer (1947) on cats and dogs, so attempts were made to adapt the iliac crest marrow aspiration procedure to the white rat. After considerable experimentation, the following successful method evolved and was used in this investigation.

The rat was lightly etherized, placed on the operating table with dorsum presented and the extremities extended. The hair over the sacral region was clipped and the skin washed first with water-detergent, then with 70 per cent alcohol, dried, and painted with tincture of Merthiolate U. S. P. (Lilly 1:1000). The anterior superior border of the iliac crest was then outlined with the fingers of the left hand. A sterile 20 gauge Quincke spinal tap needle, about 1½ inches long, was then inserted through

the skin and the muscle close to the iliac crest. Upon reaching the periosteum, the needle was simultaneously pushed and rotated with a boring motion until the needle was firmly imbedded in the bone. A suden "give" was not experienced as in sternal biopsy when the needle enters the medullary cavity of the bone. The stylet was removed and a 10 ml. syringe, that had been previously rinsed in a 3.8 per cent sodium citrate solution, was attached to the needle and 0.2 ml. of marrow was aspirated. The needle and the attached syringe were withdrawn and a small drop of marrow was expelled on each of three or four clean glass slides. Films were drawn after the manner of making peripheral blood smears. It was found that one of the shorter sides of a hemocytometer cover glass made an ideal spreader. The narrow band of smeared marrow thus made it possible to maneuver all portions of the slide under the oil immersion objective with the mechanical stage.

It is possible to fracture the ilium by aspiration technique; care was taken not to apply excess pressure to the needle. Repeat marrow aspirations were possible in 7 to 10 days.

In preliminary trials the marrow films were stained with Wright's stain. This was found not to be suitable for the purpose of the present experiments because of insufficient nuclear differentiation. The Giemsa method was then tried with a fixation of 15 minutes in methyl alcohol C. P., followed by Giemsa (1 drop of stain to 1 cc. of neutral distilled water) for one hour. Again, the technique was not completely satisfactory. Schilling (1929) reported in his monograph that Wright's stain was just as efficient for marrow staining when combined with Giemsa as was the May-Grunawald and Giemsa technique used routinely by German

hematologists. So, from this information the following technique was evolved and proved so satisfactory that it was used throughout this investigation.

1. Wright's stain..... 1 min.
2. Phosphate buffer, pH. 6.4..... 5 min.
3. Wash in beaker of neutral distilled water until proper color.
4. Air dry on end.
5. Giemsa (2 ml. of Giemsa to 1 ml. of neutral distilled water) ..... 45 min.
6. Wash in beaker of neutral distilled water until proper color.
7. Dry on end and examine.

The films were then examined under lower power (100x) and the number of megakaryocytes per 100 low-power fields were counted and noted. Marrow smears were always made three days after the main experimental regimen had been completed. Data from these smears will be referred to in the future as the post-experimental megakaryocyte count.

D. Splenectomy

Preliminary to the present investigation, it was necessary to find a successful operative procedure for removal of the spleen from the rat. Ingle and Griffith (1949) published a short description on this surgical procedure on small laboratory animals. Their method was tried in a pilot experiment but was found to be unsatisfactory because the plexiform nature of the arterial supply to the spleen in the two-to-three-month-old rat made hemostatic control very difficult. An electric cautery unit was obtained and a new pilot experiment set up to perfect a surgical technique. From these trials the following successful procedure was developed and was used throughout this investigation.

The animal was first etherized in a jar, then maintained in surgical anesthesia with a small ether cone. At the point of complete relaxation, the rat was fastened to the operating board with ventrum presentation. The hair on the ventral surface was clipped and the skin washed with water and detergent followed by 70 per cent alcohol. The surgical area was painted with tincture of Merthiolate U. S. P. (Lilly 1:1000). The cautery was turned to cutting temperature and a longitudinal incision, about an inch in length, was made through the skin and muscle layers starting just below the lower left costal border. Spleen and pancreas were then delivered through the incision. Care was taken not to traumatize the pancreas which, in the rat, is in close apposition to the spleen. The cautery was turned down to coagulating temperature and the pancreatico-splenic vessels were severed. No tying off was necessary with this method inasmuch as the cautery automatically seals the vessels as it separates them. The pancreas was quickly replaced and the muscle wall sutured with catgut, number-000, and a small curved needle. Small skin clips were used to close the skin incision. The operative area was cleansed of excess blood and body fluids, and tincture of Merthiolate was applied to the surface of the incision, especially to the cut edges. The rat was placed on clean shavings in a small, heated recovery cage for two hours. A newly operated animal was never replaced in a cage with unoperated rats. The sham-splenectomized animals went through this same procedure with the exception that the spleen, in this case, was only lifted up, then replaced and the incision closed.

Operated animals were watched carefully for four days, at which time the skin clips were removed if proper healing and repair had taken

place. Often the animal removed the clips after formation of granulation tissue had loosened them. Care was taken to be sure that the animal had completely recovered from surgery, usually after about ten days, before putting it on an experimental regimen. Less than 0.5 per cent of the operated rats failed to recover from this surgery and were not used in the experiments.

V. EXPERIMENTAL

A. Saline Pilot Experiment

1. Procedure

Before attempting to determine the effects of epinephrine, ACTH and cortisone acetate upon the blood platelets and eosinophils of the white rat, it was considered advisable to learn whether handling, anesthetizing and the injecting of solutions intra-peritoneally would produce significant effects upon the afore mentioned blood elements of these animals.

Eight, two-month-old albino rats, weighing 210 ± 10 grams were used for this experiment. After base counts were taken, four animals were splenectomized and four were left intact. Ten days were allowed for recovery from surgery before starting the experimental regimen. The time of the injection of 1 ml. of physiological saline and the immediate withdrawl of the first blood sample for platelet and eosinophil counts was designated as 0-hour. Following this, platelet and eosinophil counts were made at 15, 30, 60, 120 and 240 minute periods.

In this experiment 0-hour for each group was 8:00 a.m., with all counts completed by 12:00 a.m. to obviate any diurnal eosinophil or platelet deviations.

2. Results

a. The splenectomized group. The base eosinophil mean was 140 cells per cu. mm., and the base platelet mean was 615,000 per cu. mm. The 0-hour means were 444 eosinophils per cu. mm. and 1,143,000 platelets per cu. mm. Fifteen minutes after an intra-peritoneal injection of 1 cc. of physiological

saline, the eosinophil mean 438 cells per cu. mm., and the platelet mean was 1,130,000 per cu. mm. At the 30 and 60 minute periods the eosinophils showed means of 432 and 400 cells, respectively. Comparative stability was also shown during this period by the platelets, with means of 1,100,000 and 1,120,000 per cu. mm., respectively. At the two hour period, the eosinophil mean fell 49 per cent to 224 per cu. mm.; while the platelet mean remained relatively stable at 1,050,000 per cu. mm., only eight per cent below the 0-hour mean. At the four hour period, there was a decisive drop in the eosinophil mean to 86 cells per cu. mm. The platelet mean, only slightly disturbed, was now 1,245,000 per cu. mm., still only eight per cent below the 0-hour mean.

b. The intact group. The base mean counts on these animals were 161 eosinophils per cu. mm. and 576,000 platelets per cu. mm. At 0-hour the eosinophil mean was 208 per cu. mm., and the platelet mean was 633,000 per cu. mm. Fifteen minutes after an injection of 1 cc. of physiological saline the eosinophil mean was 189 per cu. mm. and the platelet mean was 608,000 per cu. mm. At the 30 minute period the eosinophil mean was 174 per cu. mm.; the platelet mean, 608,000 per cu. mm. At the 60 minute period, the eosinophils were 29 per cent below 0-hour with a mean count of 147 cells per cu. mm. The platelets still maintained an even keel with a mean of 598,000 per cu. mm., only three per cent below 0-hour. At the two hour point, the eosinophils dropped decisively to a mean of 117 per cu. mm. or 43 per cent below 0-hour. No change was noted in the platelet value at this point. At the four hour period the eosinophils

had dropped still farther, 61 per cent below the 0-hour level, only 80 cells per cu. mm. The platelet value was still relatively unchanged at the four hour period, showing a mean of 655,000 per cu. mm., only three per cent above the 0-hour mean.

The results are summarized in Table 1 and shown graphically in Figure 1.

B. Epinephrine Experiment

1. Procedure

Thirty-eight healthy, two-month-old albino rats, weighing 200 ± 20 grams, were used in this experiment. The objective of the experiment was to find the effect of 0.25 ml. of epinephrine (1:10,000), injected intraperitoneally, upon the blood platelets, eosinophils and marrow megakaryocytes of the splenectomized, sham-splenectomized and intact white rat. Because of the short pharmacological effect of epinephrine (Sollmann, 1942) the experimental design had to be such as to allow as many samplings as possible within a sixty minute period. The time of injection was concurrent with the taking of the first blood sample for platelet and eosinophil counts and was designated as 0-hour. Further blood samples were taken and counts made 15, 30 and 60 minutes post 0-hour.

Base counts (not to be confused with 0-hour counts) of eosinophil, platelet and megakaryocyte were taken before splenectomy and sham-splenectomy were performed. Twenty animals were then splenectomized; 12, sham-splenectomized; and six, left intact as control animals. After a recovery period of ten days the experimental regimen was started.

Table 1. Short Term Effect of Intraperitoneal Injection of Physi
Eosinophil Counts of Splenectomised and Intact Control

Animal No. and group	Base Count Pre-Op. Eosins	Plates	0-hour[4] Eosins	Plates	15-min. Eosins	Plates	30-min. Eosins	Plates
Splenect.								
14 - 1F	119	670	563	1100	474	1250	504	1200
15 - 1F	156	540	438	1120	463	1070	509	1000
16 - 1F	163	620	401	1150	413	1200	376	1120
00- 1F	125	630	376	1200	401	1000	338	1120
No. animals-4								
Min.	119	540	376	1100	401	1000	338	1000
Max.	163	670	563	1200	474	1250	509	1200
Mean	140	615	444	1143	438	1130	432	1110
% Change[5]			+217	+85	-1	-1	-1	-2
Intact								
1 - M	175	540	188	610	169	530	156	470
2 - M	181	500	181	580	175	600	138	580
3 - M	163	640	213	620	188	600	188	640
4 - M	125	700	250	720	225	700	213	740
No. animals-4								
Min.	125	500	181	580	169	530	138	470
Max.	181	700	250	720	225	700	213	740
Mean	161	576	208	633	189	608	174	608
% Change			+29	+9	-8	-3	-16	-3

[1] Dose, 1 cc. injected intraperitoneal.

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 210 ± 10 grams.

[4] Ten to fifteen days post-splenectomy; ten days post-base count in intact ra

[5] $\frac{\text{0-hour} - \text{15 min., etc.}}{\text{0-hour}}$ = % change.

f Intraperitoneal Injection of Physiological Saline[1] on Platelet[2] and
f Splenectomised and Intact Control Rats[3].

15-min.		30-min.		60-min.		2-hour		4-hour	
Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates
474	1250	504	1200	588	1200	281	980	63	1340
463	1070	509	1000	313	1130	163	1050	75	1120
413	1200	376	1120	363	1000	200	1040	94	1250
401	1000	338	1120	326	1150	250	1120	112	1270
401	1000	338	1000	313	1000	163	980	63	1120
474	1250	509	1200	588	1200	281	1120	112	1340
438	1130	432	1110	400	1120	224	1050	86	1245
-1	-1	-1	-2	-7	-1	-49	-8	-80	+8
169	530	156	470	125	530	94	640	63	660
175	600	138	580	125	620	119	640	75	620
188	600	188	640	150	540	125	520	94	580
225	700	213	740	188	700	131	740	88	760
169	530	138	470	125	530	94	520	63	580
225	700	213	740	188	700	131	740	94	760
189	608	174	608	147	598	117	635	80	655
-8	-3	-16	-3	-29	-3	-43	0	-61	+3

mber per cu. mm.

en days post-base count in intact rats.

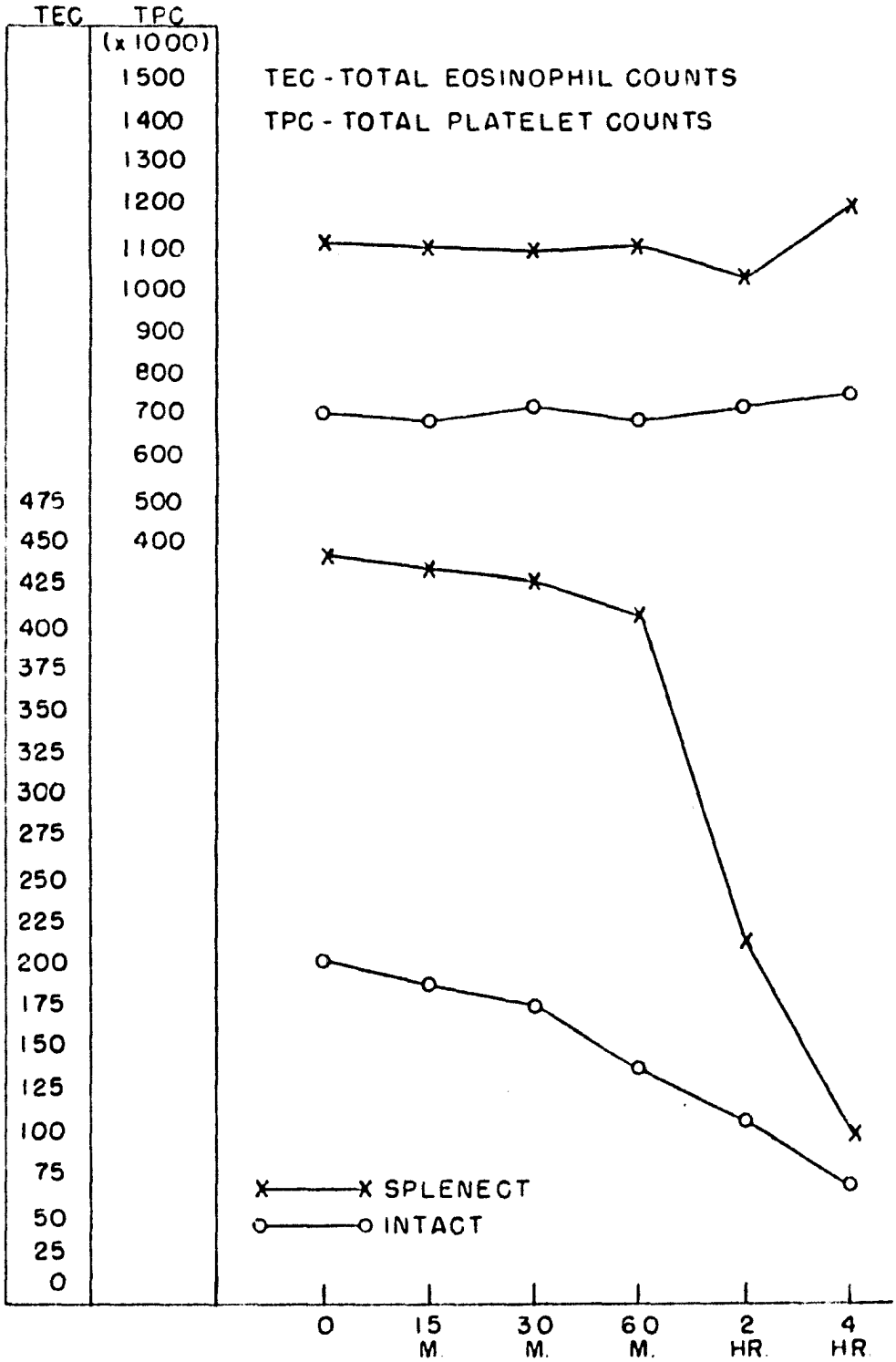

FIGURE I. EFFECT OF SALINE INJECTION ON EOSINOPHIL AND PLATELET COUNTS OF THE SPLENECTOMIZED AND INTACT RATS.

2. Results

a. The splenectomized group. The mean base count on these splenectomized animals was 171 eosinophils and 738,000 platelets per cu. mm. The megakaryocyte mean was 14.4 cells per 100 low power fields. Ten days post-splenectomy, at 0-hour, the eosinophil mean was 250 per cu. mm. and the platelet mean was 1,447,000 per cu. mm., a rise of 46 and 85 per cent, respectively. Fifteen minutes post 0-hour, the eosinophils dropped to a mean of 157 per cu. mm., and the platelets rose to a mean count of 1,822,000 per cu. mm., a decrease of 43 per cent for the eosinophils and an increase of 21 per cent for the platelets. At the thirty minute period, the eosinophils continued to drop, now showing 80 per cu. mm., or a drop of 59 per cent; while the platelets continued a rise to a mean of 1,940,000 per cu. mm., or a 24 per cent rise above 0-hour. At the sixty minute period the eosinophils had decreased further, now showing only 46 cells per cu. mm.; while the platelets had discontinued their upward climb, leveling off to a mean of 1,578,000 per cu. mm. The post-experimental megakaryocyte count showed a mean of 95 cells per 100 low power fields, an increase of 577 per cent above the pre-experimental mean.

b. The sham-splenectomized group. The mean base counts on this group were 138 eosinophils and 845,000 platelets per cu. mm. At 0-hour the eosinophil mean was 220 per cu. mm., and the platelet mean was 742,000 per cu. mm. Fifteen minutes post 0-hour the eosinophil mean dropped to 153 cells per cu. mm., while the platelet mean rose to 1,256,000 per cu. mm. This was a 31 per cent drop for the eosinophils and a 69 per cent increase for the platelets. At the thirty minute period, the eosinophils were still

dropping, now to a mean 78 cells per cu. mm., or 63 per cent below 0-hour; the platelet mean also dipped slightly to a mean of 1,230,000 per cu. mm. but still 66 per cent above 0-hour. At the sixty minute period the eosinophil mean had reached a low of 45 per cu. mm. or 80 per cent below 0-hour, and the platelets had dropped sharply to a mean of 946,000 per cu. mm., at which level they were only 27 per cent above 0-hour.

c. The intact control group. The base counts on this group were 121 eosinophils and 988,000 platelets per cu. mm. At 0-hour there were 247 eosinophils and 728,000 platelets per cu. mm. Fifteen minutes after epinephrine, the eosinophil mean dropped to 146 per cu. mm. and the platelets rose to a mean of 1,207,000 per cu. mm., a 40 per cent decrease for the eosinophils and a 65 per cent increase for the platelets. At the thirty minute period, the eosinophil mean dropped further to 74 cells per cu. mm., and the platelet mean leveled off to a mean of 1,046,000 per cu. mm. The platelets were still 43 per cent above 0-hour, however. At the sixty minute period, the eosinophils were down to a mean 53 cells per cu. mm. or 78 per cent below the 0-hour count. The platelet mean was within 12 per cent of the 0-hour level with a mean of 821,000 cells per cu. mm.

All these data are tabulated in Tables 2, 3, and 4, and are shown graphically in Figure 2.

C. Short-term ACTH Experiment

1. Procedure

Thirty-two healthy, two-month-old albino rats, weighing 220 ± 10 grams, were used for this experiment. After base eosinophil, blood platelet and marrow megakaryocyte counts had been taken, 16 rats were

Table 2. Short Term Epinephrine[1] Effect on Platelet[2], Eosinophil and Marro
Rat[3].

Animal No. and group	Base count Pre-op. Eosins	Pre-op. Plates	0-hour[4] Eosins	0-hour Plates	15-min. Eosins	15-min. Plates	30-min. Eosins	30-min. Plates
1 AM	194	664	313	1570	250	1964	125	2212
2 AM	200	752	306	1482	181	2086	75	1850
3 AM	162	676	281	1370	194	1766	81	1968
4 AM	188	572	288	1550	163	1872	69	2206
9 AM	162	924	250	1410	131	1958	63	2100
0 AM	181	984	200	1508	137	1978	57	2104
1 DF	125	682	250	1496	182	1804	125	2212
2 DF	163	664	225	1508	163	1920	75	1968
3 DF	156	734	238	1520	131	1760	88	1350
4 DF	150	626	175	1550	106	1968	75	1640
9 DF	156	946	194	1310	131	1736	69	1968
0 DF	206	928	363	1480	157	1586	125	1792
00 DF	163	776	250	1410	153	1672	88	1860
1 DM	163	884	302	1408	194	1880	75	2060
2 DM	125	900	200	1402	131	1768	63	1934
3 DM	238	696	256	1456	188	1964	50	1854
4 DM	169	890	238	1374	113	1584	75	1778
9 DM	206	924	213	1358	125	1648	50	2086
0 DM	150	748	263	1418	194	1872	125	1960
00 DM	163	794	200	1363	125	1680	75	1890
No. of animals-20								
Min.	125	572	175	1310	106	1584	50	1350
Max.	238	984	363	1570	250	2086	125	2212
Mean	171	783	250	1447	157	1822	80	1940
% Change[5]			+46	+85	-43	+21	-59	+24

[1] Dose, 0.25 ml. (1:10,000), Parke, Davis & Co., intraperitoneal.

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 200 ± 20 grams.

[4] Ten days post-splenectomy.

[5] $\frac{\text{0-hour} - \text{15 min., etc.}}{\text{0-hour}}$ = % change.

ffect on Platelet[2], Eosinophil and Marrow Megakaryocyte Counts of the Splenectomised

s	15-min. Eosins	15-min. Plates	30-min. Eosins	30-min. Plates	1-hour Eosins	1-hour Plates	Megakaryocytes Pre-Exp. /100 L.P.F.	Megakaryocytes Post-Exp. /100 L.P.F.
)	250	1964	125	2212	50	1670	31	126
!	181	2086	75	1850	56	1500	34	114
)	194	1766	81	1968	63	1480	26	82
)	163	1872	69	2206	58	1662	10	110
)	131	1958	63	2100	25	1522	16	94
3	137	1978	57	2104	25	1582	12	88
5	182	1804	125	2212	50	1608	14	100
3	163	1920	75	1968	36	1540	15	76
)	131	1760	88	1350	38	1300	37	91
)	106	1968	75	1640	25	1520	34	84
)	131	1736	69	1968	38	1484	16	96
)	157	1586	125	1792	75	1670	19	103
)	153	1672	88	1860	28	1624	18	98
8	194	1880	75	2060	63	1836	20	80
2	131	1768	63	1934	50	1608	22	76
6	188	1964	50	1854	38	1480	17	104
4	113	1584	75	1778	31	1506	12	84
8	125	1648	50	2086	25	1792	32	93
8	194	1872	125	1960	75	1660	19	99
3	125	1680	75	1890	63	1500	28	71
50	106	1584	50	1350	25	1300	10	71
70	250	2086	125	2212	75	1836	37	126
47	157	1822	80	1940	46	1578	14	95
35	-43	+21	-59	+24	-81	+6		+577

Davis & Co., intraperitoneal.

for number per cu. mm.

Table 3. Short Term Epinephrine[1] Effect on Platelet[2] and Eosinophil Counts of the Sham-Splenectomized Rat[3].

Animal No. and group	Base Count Pre-Op.		0-hour[4]		15 min.		30 min.		1 hour	
	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates
1 ME	88	994	225	620	150	1280	63	1020	38	848
2 ME	75	922	181	760	131	1468	50	1206	25	1088
3 ME	125	960	244	740	175	1242	75	1074	50	908
4 ME	106	964	175	804	138	1060	125	1008	63	872
9 ME	75	984	256	794	169	1380	88	1220	63	1054
0 ME	125	734	206	720	131	1280	44	1160	31	894
1 FE	206	852	306	794	175	1250	56	1214	38	820
2 FE	125	732	163	740	100	1186	94	1580	31	886
3 FE	150	670	219	640	150	1120	75	1208	50	900
4 FE	194	746	238	760	194	1290	125	1244	75	848
9 FE	175	844	250	804	188	1340	75	1260	31	1040
0 FE	213	734	175	728	138	1180	69	1576	50	1188
Total animals-12										
Min.	75	670	163	620	100	1060	44	1008	25	820
Max.	213	994	306	804	194	1468	125	1580	75	1188
Mean	138	845	220	742	153	1256	78	1230	45	946
% change[5]			+60	+14	-31	+ 69	-63	+66	-80	+27

[1]Dose - 0.25 ml. (1:10,000), Parke, Davis & Co., intraperitoneal.
[2]Multiply platelet values by 1000 for number per cu. mm.
[3]Weight, 200 ± 20 grams.
[4]Twelve days post-operative.
[5]$\frac{\text{0-hour - 15 min., etc.}}{\text{0-hour}}$ = % change.

Table 4. Short Term Epinephrine[1] Effect on Platelet[2] and Eosinophil Counts of the Intact Rat[3].

Animal No. and group	Base count Pre-Op. Eosins	Base count Pre-Op. Plates	0-hour[4] Eosins	0-hour[4] Plates	15-min. Eosins	15-min. Plates	30-min. Eosins	30-min. Plates	1-hour Eosins	1-hour Plates
2 GF	194	960	250	640	138	1264	63	1040	38	836
3 GF	125	980	225	760	163	1180	88	1068	75	788
4 GM	94	994	281	612	131	1030	75	1006	50	780
9 GM	106	1004	269	728	150	1136	63	988	38	752
0 GM	144	978	263	820	138	1312	81	1040	50	926
00 GM	163	1012	194	712	156	1330	75	1136	63	844
No. of animals-6										
Min.	94	960	194	612	131	1030	63	988	38	752
Max.	194	1012	281	820	163	1330	88	1136	75	926
Mean	121	988	247	728	146	1207	74	1046	52	821
% change[5]			+104	-16	-40	+65	-70	+43	-72	+12

[1] Dose, 0.25 ml. (1:10,000), Parke, Davis & Co., intraperitoneal.

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 200 ± 20 grams.

[4] Twelve days post base count.

[5] $\frac{\text{0-hour} - \text{15 min., etc.}}{\text{0-hour}}$ = % change.

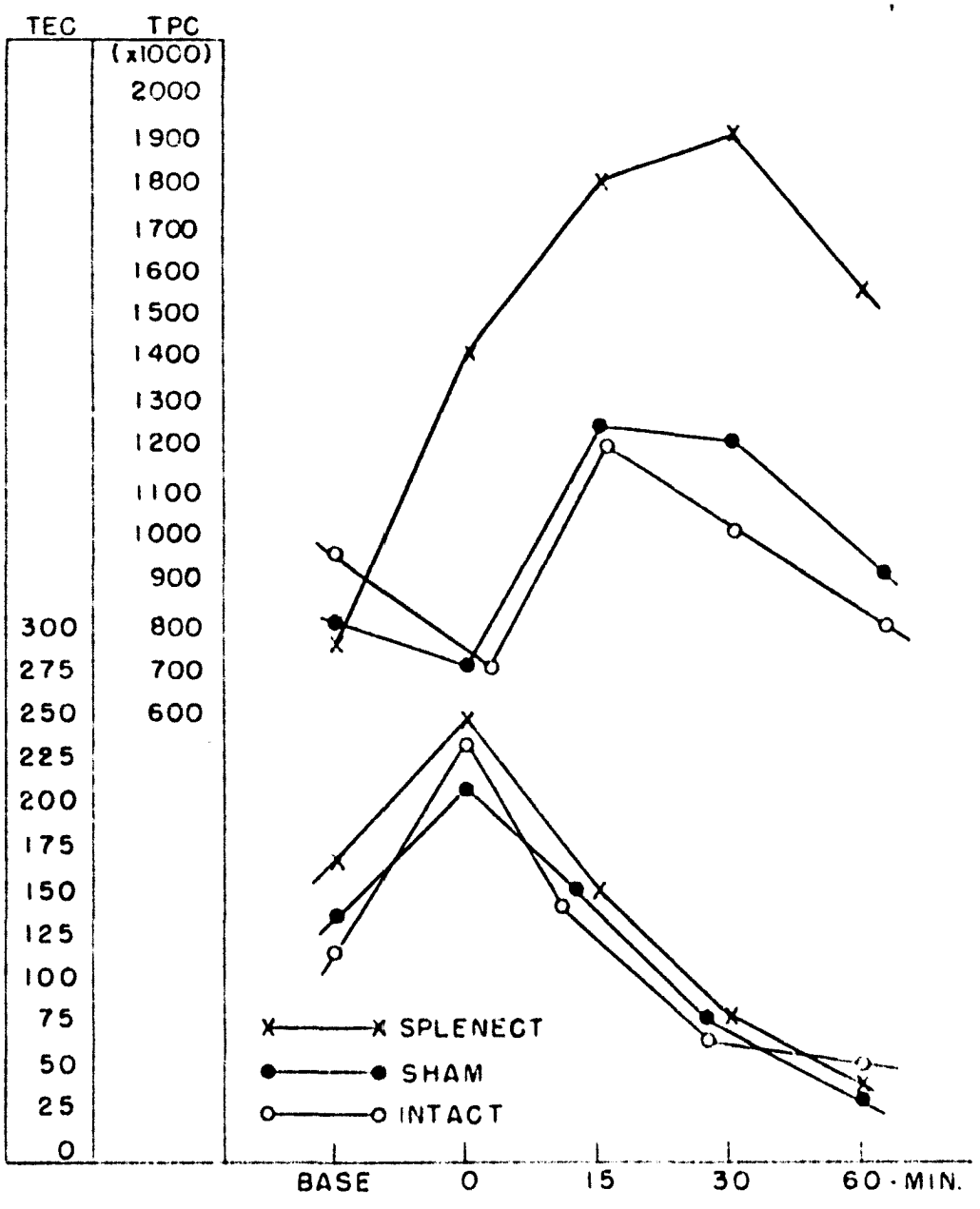

FIGURE 2. EFFECT OF EPINEPHRINE ON PLATELET AND EOSINOPHIL COUNTS OF THE SPLENECTOMIZED, SHAM-SPLENECTOMIZED AND INTACT WHITE RATS.

splenectomized; 16 were left intact as control animals. The objective of the experiment was to find the effect of a single physiological dose of ACTH upon the eosinophil, platelet and megakaryocyte counts of the splenectomized and intact rat within a short (ten hours) of time. The lengthening of the experimental time span to ten hours rather than one hour as in the epinephrine experiment was necessary because the purified ACTH preparation available was suspended in a gel medium which would tend to slow down and prolong the action of the drug. Therefore, the counts made from the sample taken concurrently with the subcutaneous injection of two Armour units per 100 grams of body weight were designated as 0-hour. Counts were subsequently made 1, 2, 3, 4, and 10 hours from 0-hour.

2. Results

a. The splenectomized group. The base mean counts on the sixteen animals in this group were 145 eosinophils and 720,000 platelets per cu. mm. The pre-experimental megakaryocyte value was 51 cells per 100 low power fields. At 0-hour, the mean eosinophil count was 157 per cu. mm. and the platelet count was 1,124,000 per cu. mm. Splenectomy had caused a 56 per cent increase in the platelet values of the group. One hour from 0-hour, the eosinophil mean was 110 per cu. mm. and the platelet mean was 845,000 per cu. mm., a drop of 29 and 33 per cent, respectively. At the two hour period, both eosinophil and platelet means were still dropping, eosinophils 65 per cu. mm. and platelets 576,000 per cu. mm. This constituted a decrease of 58 per cent for eosinophils and 48 per cent for the platelets from the 0-hour mean.

At the three hour period, the eosinophils were still dropping, having reached a mean of 29 per cu. mm., a drop of 81 per cent below the 0-hour

level. The platelets leveled off slightly at the three hour mark, showing a mean of 588,000 per cu. mm. or 47 per cent below 0-hour. The four hour values were: eosinophils, 10 per cu. mm. or a 93 per cent decrease, and platelets, 713,000 per cu. mm. or 35 per cent below 0-hour. At the ten hour period, the eosinophils were still showing a mean of 10 per cu. mm. The platelets, however, had risen slightly to a mean of 812,000 per cu. mm., or 27 per cent below 0-hour.

Post-experimental marrow megakaryocyte counts revealed a mean 83 cells per 100 low power fields or an increase of 62 per cent above the pre-experimental value.

b. The intact control group. The base mean values on the intact control group were 143 eosinophils and 790,000 platelets per cu. mm. The pre-experimental mean megakaryocyte count was 52 cells per 100 low power fields. At 0-hour the eosinophil mean was 105 per cu. mm. and the platelet mean was 817,000 per cu. mm. One hour after ACTH injection, the eosinophils had dropped to a mean of 77 per cu. mm. or a decrease of 26 per cent below 0-hour; and the platelets had dropped only slightly, to 715,000 per cu. mm., a decrease of 12 per cent from 0-hour. At the two hour period, the eosinophils were down to 53 per cu. mm., a decrease of 49 per cent; whereas, the platelets had dropped to 580,000 per cu. mm., a decrease of 29 per cent.

At the three hour period, the eosinophils were still dropping, now to a mean of 31 per cu. mm., a decrease of 70 per cent from 0-hour. The platelet mean also dropped to 444,000 per cu. mm., a decrease of 44 per cent below the 0-hour mean. At the four hour period, the eosinophil mean stood at 11 per cu. mm., 89 per cent below the 0-hour mean. The blood platelets

discontinued their downward trend at the four hour period, rising slightly to a mean of 485,000 per cu. mm.; however, this was still 41 per cent below the level of 0-hour. At the ten hour period, the eosinophils had fallen further to a mean of 7 cells per cu. mm. or 93 per cent below the 0-hour mean, and the platelets rose again, slightly, to a mean of 551,000 per cu. mm. so that at the ten hour period they stood 32 per cent below 0-hour.

The final post-experimental marrow megakaryocyte count was 88 cells per 100 low power fields, a post-experimental increase of 68 per cent over the pre-experimental mean value.

These data are summarized in Tables 5 and 6 and are shown graphically in Figure 3.

D. Long-term ACTH Experiment

1. Procedure

Nineteen healthy, two-month-old albino rats, weighing 245 ± 40 grams were used for this experiment. The objective of the experiment was to find what effect daily subcutaneous injections of two Armour units of ACTH per 100 grams of body weight would have upon daily platelet and eosinophil counts of the splenectomized and intact rat. In preparation for this part of the investigation base platelet, eosinophil and marrow megakaryocyte counts were taken on all animals. Fourteen animals were then splenectomized, and five were left intact as control animals. 0-hour was 8:00 a.m. the first day of the experiment when the first injection was given and blood samples taken for both platelet and eosinophil counts. Ten hours post 0-hour all animals were again sampled for eosinophils and

Table 5. Short Term Effect of ACTH[1] on Platelet[2], Eosinophil and Marrow Mega

Animal No. and group	Base count Pre-Op. Eosins	Pre-Op. Plates	0-hour[4] Eosins	0-hour Plates	1-hour Eosins	1-hour Plates	2-hour Eosins	2-hour Plates	3-hour Eosins	3-hour Plates
1 - 1	244	780	200	1150	138	840	63	510	25	680
3 - 1	181	810	156	1100	112	760	63	480	13	890
4 - 1	125	880	125	990	88	630	38	520	25	770
5 - 1	125	500	150	1240	75	860	44	610	19	760
1 - 2	150	860	200	1470	250	1200	94	670	25	600
3 - 2	125	860	169	1130	150	980	81	690	50	560
4 - 2	131	780	175	1100	125	1050	38	510	19	670
5 - 2	200	840	206	1120	138	1150	88	490	63	590
0 - 3	112	870	100	1240	63	1100	44	520	31	600
3 - 3	138	800	88	1250	75	900	50	600	19	810
6 - 3	156	510	131	1400	100	1240	63	890	31	760
11 - 3	119	750	112	1310	81	800	81	630	44	890
1 - 6B	112	800	156	850	94	540	94	500	44	300
3 - 6B	131	760	206	1060	88	510	69	320	38	680
4 - 6B	125	920	125	700	75	360	38	600	13	240
0 - 6B	94	810	200	870	106	600	75	680	0	360
No. of animals-16										
Min.	94	500	88	700	63	360	38	320	0	240
Max.	244	920	206	1470	250	1240	94	890	63	890
Mean	145	720	157	1124	110	845	65	576	29	588
% Change[5]			+8	+56	-29	-33	-58	-48	-81	-47

[1] Dose, 2 Armour units, purified ACTH, per 100 grams body weight, subcutaneous.

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 220 ± 10 grams.

[4] Seven to fourteen days post-operative.

[5] $\frac{\text{0-hour - 1-hour, etc.}}{\text{0-hour}}$ = % change.

1 Platelet[2], Eosinophil and Marrow Megakaryocyte Counts of the Splenectomized Rat[3].

	2-hour		3-hour		4-hour		10-hour		Megakaryocytes	
									Pre-Exp.	Post-Exp.
Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	/100 L.P.F.	/100 L.P.F.
840	63	510	25	680	0	720	0	800	63	66
760	63	480	13	890	6	930	6	840	78	60
630	38	520	25	770	13	840	0	900	26	71
860	44	610	19	760	6	890	2	960	88	63
1200	94	670	25	600	13	730	0	1200	36	69
980	81	690	50	560	6	620	25	880	41	109
1050	38	510	19	670	0	740	0	920	55	100
1150	88	490	63	590	13	810	6	1040	30	118
1100	44	520	31	600	0	720	0	1010	51	120
900	50	600	19	810	0	1100	38	600	57	146
1240	63	890	31	760	6	920	31	800	42	67
800	81	630	44	890	0	900	25	1000	63	74
540	94	500	44	300	50	300	13	420	32	60
510	69	320	38	680	25	280	6	460	41	72
360	38	600	13	240	13	490	6	560	53	78
600	75	680	0	360	0	520	0	600	58	66
360	38	320	0	240	0	280	0	420	26	60
1240	94	890	63	890	50	1100	38	1200	88	146
845	65	576	29	588	10	713	10	812	51	83
-33	-58	-48	-81	-47	-93	-35	-93	-27		462

100 grams body weight, subcutaneous.

ber per cu. mm.

Table 6. Short Term Effect of ACTH [1] on Platelet[2], Eosinophil and Marrow Megakaryocyte

Animal No. and group	Base count		0-hour [4]		1-hour		2-hour		3-hour	
	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates
1 - 5	138	880	131	750	75	760	50	480	38	500
3 - 5	112	660	138	530	94	810	44	610	19	450
11 - 5	200	670	206	660	106	640	50	390	44	520
33 - 5	181	890	94	890	75	500	63	340	38	550
1 - 6	119	690	94	780	81	920	69	560	50	420
4 - 6	200	890	81	840	88	970	75	820	38	480
5 - 6	112	960	88	940	38	980	31	610	25	360
6 - 6	125	890	100	910	44	800	50	600	13	440
5 - 7	138	660	94	820	56	600	38	580	19	410
6 - 7	131	760	88	880	106	540	63	630	25	460
11 - 7	112	700	94	940	75	540	50	520	38	430
0 - 7	156	960	75	750	50	620	44	540	25	400
1 - 8	138	920	144	860	112	640	63	610	25	410
3 - 8	150	770	100	920	88	840	63	810	38	430
4 - 8	112	820	88	820	81	740	50	700	25	480
00 - 8	175	600	63	780	50	520	44	600	25	360
No. of animals-16										
Min.	112	600	63	530	38	500	31	340	13	360
Max.	200	960	206	940	112	980	69	820	44	550
Mean	143	790	105	817	77	715	53	580	31	444
% Change[5]			-23	+3	-26	-12	-49	-29	-70	-44

[1] Dose, 2 Armour units, purified ACTH, per 100 grams body weight, subcutaneous.

[2] Multiply platelet values for number per cu. mm.

[3] Weight, 220 ± 10 grams.

[4] Six to fourteen days post base count.

[5] $\frac{\text{0-hour - 1-hour, etc.}}{\text{0-hour}}$ = % change.

[2], Eosinophil and Marrow Megakaryocyte Counts of the Intact Rat[3].

ur	2-hour		3-hour		4-hour		10-hour		Megakaryocytes	
Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	Pre-Exp. /100 L.P.F.	Post-Exp. /100 L.P.F.
760	50	480	38	500	6	430	0	490	64	63
810	44	610	19	450	25	310	0	380	32	84
640	50	390	44	520	6	390	0	300	40	62
500	63	340	38	550	0	370	0	300	50	80
920	69	560	50	420	38	480	50	520	88	67
970	75	820	38	480	44	470	19	440	76	117
980	31	610	25	360	13	440	0	640	55	114
800	50	600	13	440	6	380	13	660	30	92
600	38	580	19	410	6	460	0	520	51	87
540	63	630	25	460	13	330	6	490	57	100
540	50	520	38	430	6	500	0	580	42	96
620	44	540	25	400	0	580	0	600	32	82
640	63	610	25	410	13	560	0	740	63	97
840	63	810	38	430	6	610	6	690	41	95
740	50	700	25	480	0	730	13	790	57	87
520	44	600	25	360	0	720	0	680	44	96
500	31	340	13	360	0	310	0	300	30	62
980	69	820	44	550	44	730	50	690	88	117
715	53	580	31	444	11	485	7	551	52	88
-12	-49	-29	-70	-44	-89	-41	-93	-32		-68

r 100 grams body weight, subcutaneous.

cu. mm.

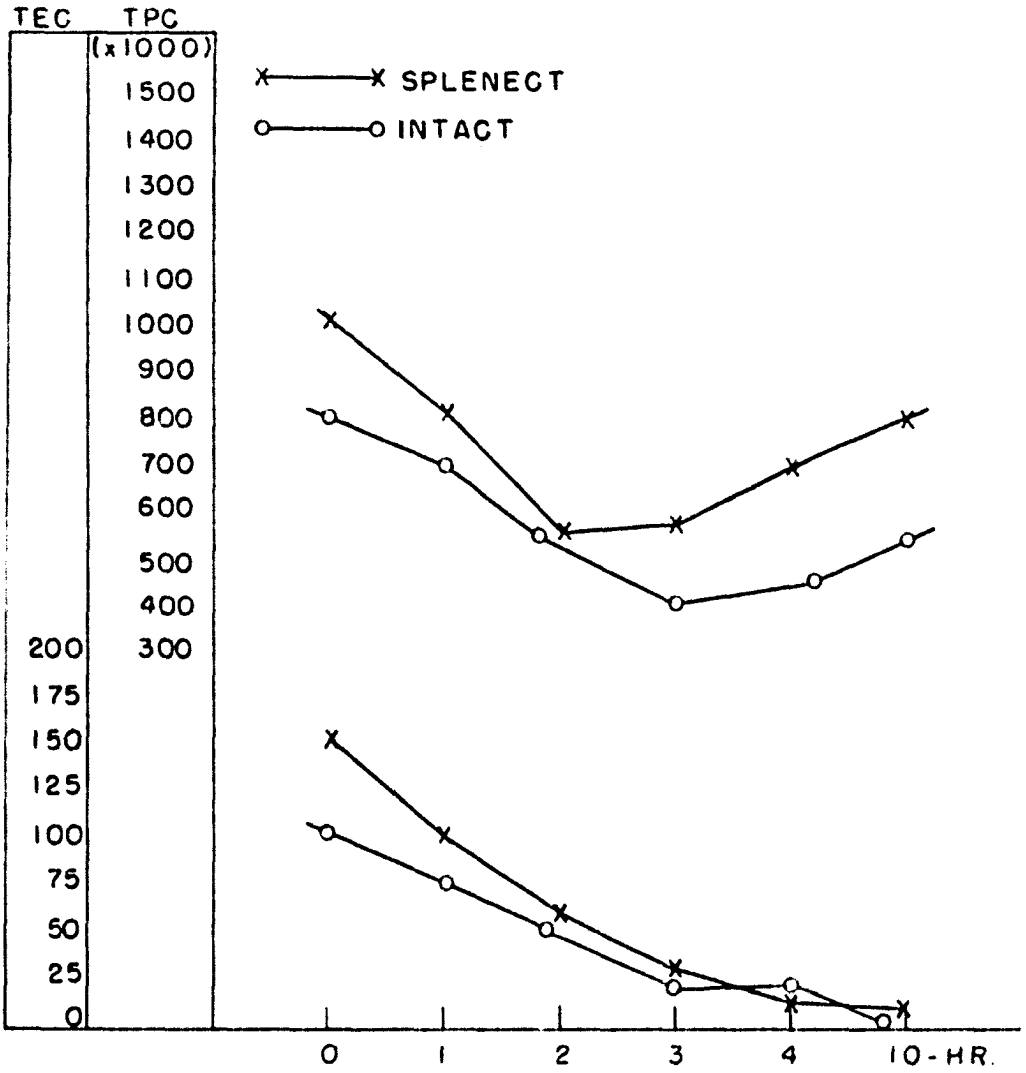

FIGURE 3. EFFECT OF ACTH ON PLATELET AND EOSINOPHIL COUNTS OF SPLENECTOMIZED AND INTACT WHITE RATS.

platelets. After the first day the animals were injected between 8:00 and 10:00 a.m. and counts were made between 1:00 and 3:00 p.m. the same day. All animals were injected daily for fourteen days, and daily eosinophil and platelet counts were made on all days except the seventh and eighth. Counts were continued on the 16th and 19th days to ascertain if there was any tapering off effect after the injections had been terminated.

The regular diet was maintained throughout and no significant weight changes were noted.

Two animals were removed from the experiment before 0-hour, one by anesthetic death and the other because it showed signs of a respiratory disease.

2. Results

a. The splenectomized group. The base mean counts on the splenectomized group were 133 eosinophils and 727,000 platelets per cu. mm. The pre-experimental megakaryocyte mean was 50 cells per 100 low power fields. At 0-hour the eosinophil mean was 133 per cu. mm., and the platelet mean was 1,220,000 per cu. mm. The first day's mean count was 23 eosinophils and 687,000 platelets per cu. mm., a drop of 82 per cent and 44 per cent, respectively. On the second day the eosinophil mean was 14 per cu. mm., and the platelet mean was back up to 1,218,000 per cu. mm. On the third day the eosinophils rose again to a mean of 26 per cu. mm. and the platelets to 1,533,000 per cu. mm., a drop of 84 per cent from 0-hour in eosinophils and an increase of 26 per cent above 0-hour in the platelet mean. On the fourth day the eosinophils went to a mean of 44 per cu. mm. and the platelets dropped to a mean of 1,245,000 per cu. mm. On the fifth day the eosinophils dropped to a mean of 12 per cu. mm., and the platelets

went up to a mean of 1,553,000 per cu. mm. The sixth day the eosinophil mean was 16 per cu. mm. and the platelet mean was 1,530,000 per cu. mm.

On the ninth day the animals were in almost complete eosinopenia with a mean of 3 per cu. mm., a drop of 98 per cent below 0-hour; while the platelets rose to a mean of 1,863,000 per cu. mm., an increase of 52 per cent above 0-hour. On the tenth, eleventh, twelfth, and thirteenth days the animals remained in eosinopenia with mean counts of 6, 5, 5, and 7 cells per cu. mm., respectively. The platelet means for the same days were: 1,740,000; 1,628,000; 1,584,000; and 1,750,000 per cu. mm., still 30 to 40 per cent above the 0-hour mean. On the fourteenth, or last day of the injections, the eosinophils presented a mean of 11 per cu. mm., or 93 per cent below 0-hour; and the platelets showed a mean of 1,664,000 per cu. mm., or 36 per cent above the 0-hour mean. Counts on the sixteenth day showed an eosinophil mean of 8 cells, still 95 per cent below the 0-hour mean and the platelet mean was 1,547,000 per cu. mm. On the nineteenth day, five days after the last injection, the eosinophils were up to a mean of 66 cells per cu. mm.; while the platelets dropped to a mean of 1,053,000 per cu. mm. This platelet value was 14 per cent below the 0-hour level.

Post-experimental marrow megakaryocyte counts gave a mean of 96 cells per 100 low power fields, or a mean increase of 92 per cent over pre-experimental value.

b. The intact group. The base means on the intact control group were 133 eosinophils and 756,000 platelets per cu. mm. The pre-experimental megakaryocyte mean on this day was 47 cells per 100 low power fields. At

0-hour the eosinophil mean was the same, 133 per cu. mm., and 754,000 platelets per cu. mm. showed no significant change from the base mean. The first day's counts gave an eosinophil mean of 23 per cu. mm., a significant drop from the 0-hour level. The first day's platelet mean showed 614,000 per cu. mm., only a slight decrease from the 0-hour value. The second days's means, eosinophils, 29 per cu. mm. and platelets, 840,000 per cu. mm., were slight changes from the preceding day's values. On the third day the eosinophils rose to 79 per cu. mm. but were still 48 per cent below the 0-hour mean, whereas the platelets took a significantly sharp rise to 1,588,000 per cu. mm. or an increase of 110 per cent above 0-hour.

On the fourth, fifth, sixth, and ninth days the eosinophils showed a steady downward trend with mean values of 39, 26, 18, and 1.2 cells per cu. mm., respectively. As noted, there was complete eosinopenia on the ninth day or a decrease of 99 per cent below the 0-hour level. On the fourth, fifth, sixth, and ninth days the platelets showed an increase to a level that was to be held for three days; starting on the fourth day with a mean of 1,260,000 per cu. mm., the fifth day with 1,464,000 per cu. mm., the sixth day with 1,388,000 per cu. mm., to the ninth day with a mean count of 1,752,000 per cu. mm. This ninth day mean was an increase of 132 per cent above 0-hour.

The eosinophil mean rose slightly but not significantly on the tenth day to 14 cells per cu. mm., but the animals remained in eosinopenia for the rest of the experiment, showing 4, 19, 23, 18, and 19 cells per cu. mm. on the eleventh, twelfth, thirteenth, fourteenth, and sixteenth days, respectively. On the nineteenth day, five days after ACTH injections had been terminated, the eosinophil mean rose to 48 cells per cu. mm. The

platelet means for the eleventh, twelfth, thirteenth, fourteenth, and sixteenth days were all still over 100 per cent above 0-hour with 1,770,000, 1,640,000, 1,596,000, 1,600,000 and 1,612,000 per cu. mm., respectively. On the nineteenth day, five days after the ACTH injections had been terminated, the platelet mean fell to 900,000 per cu. mm., only 19 per cent above the 0-hour level.

The post-experimental marrow megakaryocytes showed a mean count of 153 cells per 100 low power fields, 153 per cent above the pre-experimental base mean.

These data are tabulated in Tables 7, 8, 9, and 10 and are presented graphically in Figure 4.

E. Short-term Cortisone Acetate Experiment

1. Procedure

Thirty-two healthy, two-month-old albino rats, weightin 210 ± 10 grams were used in this experiment. The design of the experiment was similar to the short-term ACTH investigation inasmuch as it was deemed advisable to find the effect of a single physiological dose of cortisone acetate upon platelet, eosinophil and marrow megakaryocyte counts of the white rat. As this preparation was suspended in a sterile saline, instead of a gel suspension, as was ACTH, it was found unnecessary to have a long sampling period. The time of the intraperitoneal injection of 2.5 mg. per 100 grams of body weight was designated as 0-hour, and serial counts were made at 15, 30, 60, 120, and 240 minute periods.

Table 7. Long Term[1] ACTH Effect on Platelet[2] and Marrow Megakaryocyte Counts of the Sple

Animal No. and group	Base Pre-Op.	0-hour[4]	1-day	2-day	3-day	4-day	5-day	6-day	9-day	10-day	11-day	12-day
1 - 3M	580	1420	720	1120	1440	1100	1470	1500	1800	1850	1640	1600
3 - 3M	590	1470	650	850	1100	1050	1520	1480	1890	1800	1790	1800
5 - 3M	760	-- Anesthetic death --										
0 - 3M	700	-- Removed from the experiment-respiratory involvement --										
1 - 4M	860	1100	800	1250	1660	1650	1680	1650	2220	2000	1810	1790
3 - 4M	570	1380	660	1600	1460	1410	1830	1790	1940	1900	1050	1100
4 - 4M	680	1250	700	1520	1000	1400	1800	1800	1900	1890	1980	1800
5 - 4M	680	1150	720	1470	1710	1260	1420	1500	1960	1850	1640	1650
0 - 4M	740	1300	600	1670	1520	1160	1500	1550	1800	1790	1710	1500
1 - 6F	790	1100	640	1050	1670	1200	1760	1500	1950	1640	1680	1630
3 - 6F	900	1010	740	1100	1800	1140	1900	1110	1660	1050	1150	1150
4 - 6F	810	1100	710	920	1890	1210	1250	1610	1720	1740	1690	1700
5 - 6F	680	1360	670	1000	1550	1240	1100	1300	1540	1670	1720	1780
0 - 6F	700	1000	630	1050	1600	1120	1400	1560	1980	1700	1680	1510
No. of animals-14												
Min.	570	1000	600	850	1000	1050	1100	1110	1540	1050	1050	1100
Max.	900	1470	800	1600	1890	1650	1900	1800	2220	2000	1980	1800
Mean	727	1220	687	1218	1533	1245	1553	1530	1863	1740	1628	1584
% Change[5]		+68	-44	0	+26	+2	+27	+25	+52	+42	+33	+30

[1] ACTH administered daily, first to fourteenth day inclusive; dose, 2 Armour units per

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 245 ± 40 grams.

[4] 0-hour was ten days post-splenectomy.

[5] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change.

[2] and Marrow Megakaryocyte Counts of the Splenectomized Rat[3].

	5-day	6-day	9-day	10-day	11-day	12-day	13-day	14-day	16-day	19-day	Megakaryocytes Pre-Exp. /100 L.P.F.	Megakaryocytes Post-Exp. /100 L.P.F.
	1470	1500	1800	1850	1640	1600	1700	1650	1350	970	50	84
	1520	1480	1890	1800	1790	1800	1820	1750	1200	1100	62	82
-respiratory involvement --												
)	1680	1650	2220	2000	1810	1790	1800	1800	1300	1220	60	86
)	1830	1790	1940	1900	1050	1100	1790	1600	1540	950	45	120
)	1800	1800	1900	1890	1980	1800	1800	1750	1260	990	34	110
)	1420	1500	1960	1850	1640	1650	1830	1700	1750	1200	62	130
)	1500	1550	1800	1790	1710	1500	1850	1650	1250	910	49	100
)	1760	1500	1950	1640	1680	1630	1350	1400	1600	970	65	140
)	1900	1110	1660	1050	1150	1150	1520	1600	1770	1020	49	80
)	1250	1610	1720	1740	1690	1700	1770	1650	1850	1240	35	70
)	1100	1300	1540	1670	1720	1780	1970	1850	1900	1200	43	70
)	1400	1560	1980	1700	1680	1510	1800	1790	1800	870	30	76
)	1100	1110	1540	1050	1050	1100	1350	1400	1200	870	30	70
0	1900	1800	2220	2000	1980	1800	1970	1850	1900	1240	62	140
5	1553	1530	1863	1740	1628	1584	1750	1664	1547	1053	50	96
2	+27	+25	+52	+42	+33	+30	+44	+36	+26	-14		+92

enth day inclusive; dose, 2 Armour units per 100 grams body weight, subcutaneous.
ber per cu. mm.

Table 8. Long Term[1] ACTH Effect on Platelet[2] and Marrow Megakaryocyt

Animal No. and group	Base Pre-Op.	0-hour[4]	1-day	2-day	3-day	4-day	5-day	6-day	9-day	10-day	11-day	12-day
1 - 5F	690	690	600	800	1500	1300	1500	1570	1850	1690	1720	163
3 - 5F	790	790	760	940	1690	1250	1300	1260	1580	1780	1800	180
4 - 5F	890	890	590	820	1700	970	1120	1320	2030	1730	1700	150
5 - 5F	600	600	550	800	1600	1400	1800	1560	1620	1930	1890	176
0 - 5F	810	800	570	840	1450	1380	1600	1230	1680	1600	1740	151
No. of animals-5												
Min.	600	600	550	800	1450	970	1120	1230	1580	1600	1700	150
Max.	890	890	760	940	1700	1400	1800	1570	2030	1930	1890	180
Mean	756	754	614	840	1588	1260	1464	1388	1752	1746	1770	161
% Change[5]		0	-19	+8	+110	+68	+94	+84	+132	+131	+134	+11

[1] ACTH administered daily, first to fourteenth day inclusive; dose, 2 Armour units

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 245 ± 40 grams.

[4] 0-hour was ten days post base count.

[5] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change.

H Effect on Platelet[2] and Marrow Megakaryocyte Counts of the Intact Rat[3].

4-day	5-day	6-day	9-day	10-day	11-day	12-day	13-day	14-day	16-day	19-day	Megakaryocytes Pre-Exp. /100 L.P.F.	Megakaryocytes Post-Exp. /100 L.P.F.
1300	1500	1570	1850	1690	1720	1630	1750	1730	1550	780	59	84
1250	1300	1260	1580	1780	1800	1800	1450	1500	1600	900	32	120
970	1120	1320	2030	1730	1700	1500	1600	1600	1340	970	61	172
1400	1800	1560	1620	1930	1890	1760	1680	1620	1870	1060	38	89
1380	1600	1230	1680	1600	1740	1510	1500	1550	1700	790	47	131
970	1120	1230	1580	1600	1700	1500	1500	1500	1340	780	32	84
1400	1800	1570	2030	1930	1890	1800	1750	1730	1870	1060	61	172
1260	1464	1388	1752	1746	1770	1640	1596	1600	1612	900	47	119
±68	±94	±84	±132	±131	±134	±117	±111	±112	±114	±19		±153

urteenth day inclusive; dose, 2 Armour units per 100 grams body weight, subcutaneous.

r number per cu. mm.

Table 9. Long Term[1] Effect of ACTH on Eosinophil[2] Counts of the Splenectom

Animal No. and group	Base Pre-Op.	0-hour[4]	1-day	2-day	3-day	4-day	5-day	6-day	9-day	10-day	11
1 - 3M	125	144	0	13	12	13	13	13	0	0	
3 - 3M	112	188	25	19	0	13	19	25	0	25	
5 - 3M	200										
0 - 3M	231										
1 - 4M	144	150	0	0	19	50	6	6	0	19	
3 - 4M	125	238	19	25	25	6	6	19	6	6	
4 - 4M	125	112	25	0	0	13	6	25	13	0	
5 - 4M	131	163	31	13	0	13	13	31	0	0	
0 - 4M	125	131	12	0	6	0	0	19	0	0	
1 - 6F	112	156	31	0	6	63	13	0	0	0	
3 - 6F	138	150	13	31	62	94	25	6	13	6	
4 - 6F	150	150	13	19	75	88	0	13	6	13	
5 - 6F	150	200	50	13	44	75	19	19	0	0	
0 - 6F	194	206	63	25	62	94	19	13	0	0	
No. of animals-14											
Min.	112	112	0	0	0	0	0	0	0	0	
Max.	231	238	63	31	75	94	25	31	13	25	
Mean	147	163	23	14	26	44	12	16	3	6	
% Change[5]		+10	-86	-91	-84	-73	-93	-90	-98	-96	

[1] ACTH administered daily, first to fourteenth day inclusive; dose, 2 Armour units

[2] Values given are number of cells per cu. mm.

[3] Weight, 245 ± 40 grams

[4] Ten days post-splenectomy

[5] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change

TH on Eosinophil[2] Counts of the Splenectomised Rat[3].

lay	4-day	5-day	6-day	9-day	10-day	11-day	12-day	13-day	14-day	16-day	19-day
2	13	13	13	0	0	0	0	13	6	19	75
)	13	19	25	0	25	0	0	6	6	19	63
)	50	6	6	0	19	13	6	19	19	19	75
5	6	6	19	6	6	6	13	0	0	13	106
)	13	6	25	13	0	6	6	6	0	0	94
)	13	13	31	0	0	6	0	0	13	0	75
5	0	0	19	0	0	0	0	0	6	6	50
5	63	13	0	0	0	13	13	6	19	19	75
2	94	25	6	13	6	6	0	6	13	0	31
5	88	0	13	6	13	0	13	0	0	37	69
4	75	19	19	0	0	0	0	0	19	31	25
2	94	19	13	0	0	13	13	25	26	13	69
)	0	0	0	0	0	0	0	0	0	0	25
5	94	25	31	13	25	13	13	25	26	37	106
6	44	12	16	3	6	5	5	7	11	8	66
4	-73	-93	-90	-98	-96	-97	-97	-96	-93	-95	-59

teenth day inclusive; dose, 2 Armour units per 100 grams body weight.

u. mm.

Table 10. Long Term[1] Effect of ACTH on Eosinophil[2] Counts of

Animals No. and group	Base Pre-Op.	0-hour[4]	1-day	2-day	3-day	4-day	5-day	6-day	9-day	10-d
1 - 5F	119	119	13	0	62	31	6	6	0	13
3 - 5F	125	125	19	38	75	38	31	38	0	25
4 - 5F	175	175	50	56	94	63	63	13	0	25
5 - 5F	125	125	25	31	75	25	19	13	6	0
0 - 5F	119	119	6	19	88	38	13	19	0	6
No. of animals-5										
Min.	119	119	6	0	62	25	6	6	0	0
Max.	175	175	50	56	94	63	63	38	6	25
Mean	133	133	23	29	79	39	26	18	1.2	14
% Change[5]		0	-82	-78	-48	-71	-80	-86	-99	-89

[1] ACTH administered daily, first to fourteenth day inclusive; dose, 2 Armour

[2] Values are number of cells per cu. mm.

[3] Weight, 245 ± 40 grams

[4] Ten days post base count

[5] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change

ffect of ACTH on Eosinophil[2] Counts of the Intact Rat[3].

-day	4-day	5-day	6-day	9-day	10-day	11-day	12-day	13-day	14-day	16-day	19-day
62	31	6	6	0	13	13	13	31	13	13	50
75	38	31	38	0	25	0	36	25	26	26	63
94	63	63	13	0	25	0	31	19	19	37	50
75	25	19	13	6	0	6	19	31	19	26	63
88	38	13	19	0	6	0	0	13	13	31	38
62	25	6	6	0	0	0	0	13	13	13	38
94	63	63	38	6	25	13	36	31	26	37	63
79	39	26	18	12	14	4	19	23	18	19	48
-48	-71	-80	-86	-99	-89	-97	-86	-82	-86	-86	-64

ourteenth day inclusive; dose, 2 Armour units per 100 grams body weight

m.

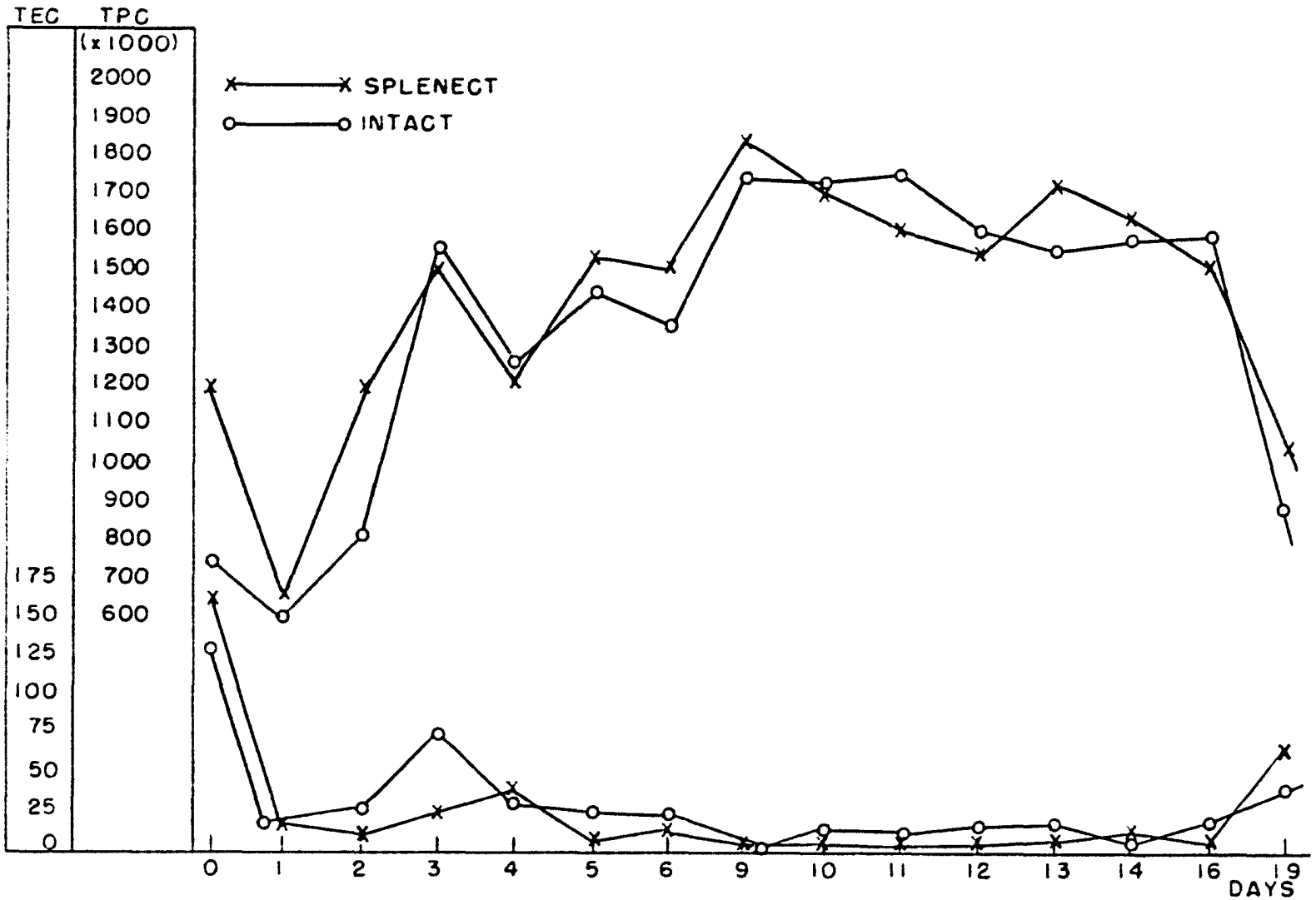

FIGURE 4. EFFECT OF DAILY ACTH ON PLATELET AND EOSINOPHIL COUNTS OF SPLENECTOMIZED AND INTACT RATS.

2. Results

a. The splenectomized group. The mean base counts on the splenectomized group were 134 eosinophils and 724,000 platelets per cu. mm. The base megakaryocyte mean was 49 cells per 100 low power fields. At 0-hour the eosinophil mean was 308 per cu. mm., and the platelet mean was 1,115,000 per cu. mm. Fifteen minutes after 0-hour the eosinophils dropped 9 per cent to a mean of 276 cells per cu. mm. The platelets rose in the same period to a mean of 1,267,000 per cu. mm., or an increase of 13 per cent. At the thirty minute period, the eosinophils had dropped again to a mean of 258 per cu. mm., and the platelets had risen just slightly to a mean of 1,277,000 per cu. mm. At the sixty minute period the eosinophils dropped sharply to a mean of 186 per cu. mm., a drop of 39 per cent from 0-hour. The platelets also dropped significantly to 42 per cent below 0-hour, now standing at 641,000 per cu. mm. At the two hour point, the eosinophils were still dropping, now 88 cells per cu. mm., or a mean decrease from 0-hour of 71 per cent. The platelets discontinued their downward trend at the two hour period and were back up to a mean of 1,181,000 per cu. mm., or 5 per cent above their 0-hour mean. At the four hour period, the eosinophil mean had dropped still farther to 11 cells per cu. mm. or 96 per cent below the 0-hour mean; whereas no significant change was noted in the platelets, now registering a mean of 1,064,000 per cu. mm., or four per cent below the 0-hour mean.

The post-experimental marrow megakaryocyte count on this group showed 74 cells per 100 low power fields, or a 51 per cent increase over the pre-experimental mean.

b. The intact group. The base counts on this group showed 143 eosinophils and 641,000 platelets per cu. mm. There was a base mean count of 46 megakaryocytes per 100 low power fields also. At the 0-hour the mean eosinophil count was 251 per cu. mm.; the platelet mean was 831,000 per cu. mm. Fifteen minutes post 0-hour the eosinophils showed a mean of 241 per cu. mm. and the platelets a mean of 879,000 per cu. mm. At the thirty minute period the eosinophils dropped to a mean of 187 cells per cu. mm., a decrease of 25 per cent from 0-hour; while the platelets were relatively undisturbed, registering a mean of 883,000 per cu. mm., or 6 per cent above 0-hour level. At the sixty minute period, both elements dropped sharply, the eosinophils to a mean of 149 per cu. mm. and the platelets to 586,000 per cu. mm. These significant decreases were 40 and 30 per cent, respectively, below the 0-hour means. The two hour period revealed a further decrease in the eosinophil mean, now down to 69 cells per cu. mm.; whereas the platelets were back up to 886,000 per cu. mm. At four hours, the eosinophils were 93 per cent below 0-hour, and the platelets had climbed to their highest point, 1,020,000 per cu. mm., or 34 per cent above the 0-hour mean.

The post-experimental megakaryocyte count showed a large increase in these cells, registering 154 megakaryocytes per 100 low power fields, or 234 per cent above the pre-experimental mean.

The results are summarized in Tables 11 and 12 and are shown graphically in Figure 5.

Table 11. Short Term Cortisone Acetate[1] Effect on Blood Platelet[2], Eosinopl
Splenectomized Rat[3].

Animal No. and group	Base Count Pre-Op. Eosins	Pre-Op. Plates	0-hour[4] Eosins	0-hour Plates	15-min. Eosins	15-min. Plates	30-min. Eosins	30-min. Plates	60-min. Eosins	60-min. Plate
1-5F	125	710	319	1300	238	1580	306	1550	206	700
3-5F	119	810	394	1100	444	1400	382	980	250	650
4-5F	125	870	250	1080	300	960	294	1350	225	460
5-5F	163	890	300	1320	351	1200	213	1220	188	680
0-5F	175	740	457	1380	401	960	376	1450	326	730
1-3M	156	860	175	980	138	1190	144	1100	144	320
3-3M	138	630	281	1030	169	1250	94	1490	94	410
4-3M	125	530	194	1000	175	1310	181	1120	125	330
5-3M	131	850	294	1120	194	1200	188	1150	81	620
0-3M	138	670	319	1410	125	1580	112	1400	94	800
1-1F	112	610	339	1170	319	1500	313	1190	269	880
3-1F	156	540	345	1210	394	1000	388	1150	188	790
11-1F	112	830	238	1400	213	1420	163	1200	138	730
13-1F	94	600	409	1100	403	1200	463	1400	275	880
No. of animals-14										
Min.	94	530	175	980	125	960	94	980	81	320
Max.	175	710	457	1410	444	1580	463	1550	326	880
Mean	134	724	308	1115	276	1267	258	1277	186	641
% change[5]			+126	+54	-9	+13	-16	+14	-39	-42

[1] Dose - 2.5 mg. in physiological saline per 100 grams of body weight, intraperi

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 210 ± 10 grams.

[4] Ten to fifteen days post-splenectomy.

[5] $\frac{\text{0-hour - 15-min., etc.}}{\text{0-hour}}$ = % change.

Effect on Blood Platelet[2], Eosinophil and Marrow Megakaryocyte Counts of the

ites	30-min. Eosins	30-min. Plates	60-min. Eosins	60-min. Plates	2-hour Eosins	2-hour Plates	4-hour Eosins	4-hour Plates	Megakaryocytes Pre-Exp. /100 L.P.F.	Megakaryocytes Post-Exp. /100 L.P.F.
580	306	1550	206	700	126	1260	0	1000	42	73
400	382	980	250	650	106	1080	6	1100	36	61
960	294	1350	225	460	138	1200	19	1060	51	91
200	213	1220	188	680	144	1120	13	1240	64	94
960	376	1450	326	730	15	900	0	1270	31	71
190	144	1100	144	320	81	1120	13	1600	26	52
250	94	1490	94	410	63	1300	38	800	34	91
310	181	1120	125	330	31	1050	13	730	42	68
200	188	1150	81	620	56	1490	6	930	96	63
580	112	1400	94	800	63	1360	0	900	25	69
500	313	1190	269	880	125	1020	25	1150	38	76
000	388	1150	188	790	88	960	13	1100	37	81
420	163	1200	138	730	112	1300	0	920	94	93
200	463	1400	275	880	88	1380	13	1120	69	88
960	94	980	81	320	15	900	0	730	25	61
580	463	1550	326	880	144	1490	38	1600	96	94
267	258	1277	186	641	88	1181	11	1064	49	74
+13	-16	+14	-39	-42	-71	+5	-96	-4		+51

100 grams of body weight, intraperitoneal.

per cu. mm.

Table 12. Short Term Cortisone Acetate[1] Effect on Blood Platelet[2], Eosinophil and

Animal No. and group	Base count Pre-Op. Eosins	Pre-Op. Plates	0-hour[4] Eosins	0-hour Plates	15-min. Eosins	15-min. Plates	30-min. Eosins	30-min. Plates	60-min. Eosins	60-min. Plates	2 Eos
1-4M	112	700	288	620	319	640	206	840	188	450	
3-4M	125	520	250	680	219	770	169	580	163	700	
4-4M	150	560	306	760	388	960	188	1070	156	460	
0-4M	119	540	225	890	194	860	169	990	125	850	
4-2F	112	610	194	1050	231	1030	244	900	213	620	1
5-2F	169	820	288	810	188	1010	138	630	138	550	
0-2F	181	510	256	920	231	890	263	970	200	640	1
11-2F	163	500	250	780	219	800	138	940	125	520	
13-2F	163	850	225	800	206	850	175	960	94	450	
14-2F	138	800	231	1000	213	980	181	950	88	620	
No. of animals-10											
Min.	112	500	194	620	188	640	138	580	88	450	
Max.	181	850	306	1050	388	1030	263	1070	213	850	1
Mean	143	641	251	831	241	879	187	883	149	586	
% Change[5]			+75	+29	-3	+5	-25	+6	-40	-30	-

[1] Dose, 2.5 mg. in physiological saline per 100 grams of body weight, intraperitoneal.

[2] Multiply platelet values by 1000 for number per cu. mm.

[3] Weight, 210 ± 10 grams.

[4] Ten to fifteen days post base count.

[5] $\frac{\text{0-hour} - \text{15-min., etc.}}{\text{0-hour}}$ = % change.

on Blood Platelet[2], Eosinophil and Marrow Megakaryocyte Counts of the Intact Rat[3].

30-min.		60-min.		2-hour		4-hour		Megakaryocytes	
								Pre-Exp.	Post-Exp.
Eosins	Plates	Eosins	Plates	Eosins	Plates	Eosins	Plates	/100 L.P.F.	/100 L.P.F.
206	840	188	450	38	460	13	860	36	83
169	580	163	700	56	560	25	1060	42	189
188	1070	156	460	63	1050	31	1020	59	367
169	990	125	850	31	700	13	1100	68	86
244	900	213	620	175	930	6	1040	48	152
138	630	138	550	31	1060	13	1100	30	120
263	970	200	640	112	1010	25	980	34	133
138	940	125	520	63	980	6	1000	24	121
175	960	94	450	69	990	13	1120	56	147
181	950	88	620	56	1120	19	1000	61	129
138	580	88	450	31	460	6	860	30	83
263	1070	213	850	175	1120	31	1120	68	367
187	883	149	586	69	886	16	1020	46	154
-25	+6	-40	-30	-72	+6	-93	+34		+234

ams of body weight, intraperitoneal.

cu. mm.

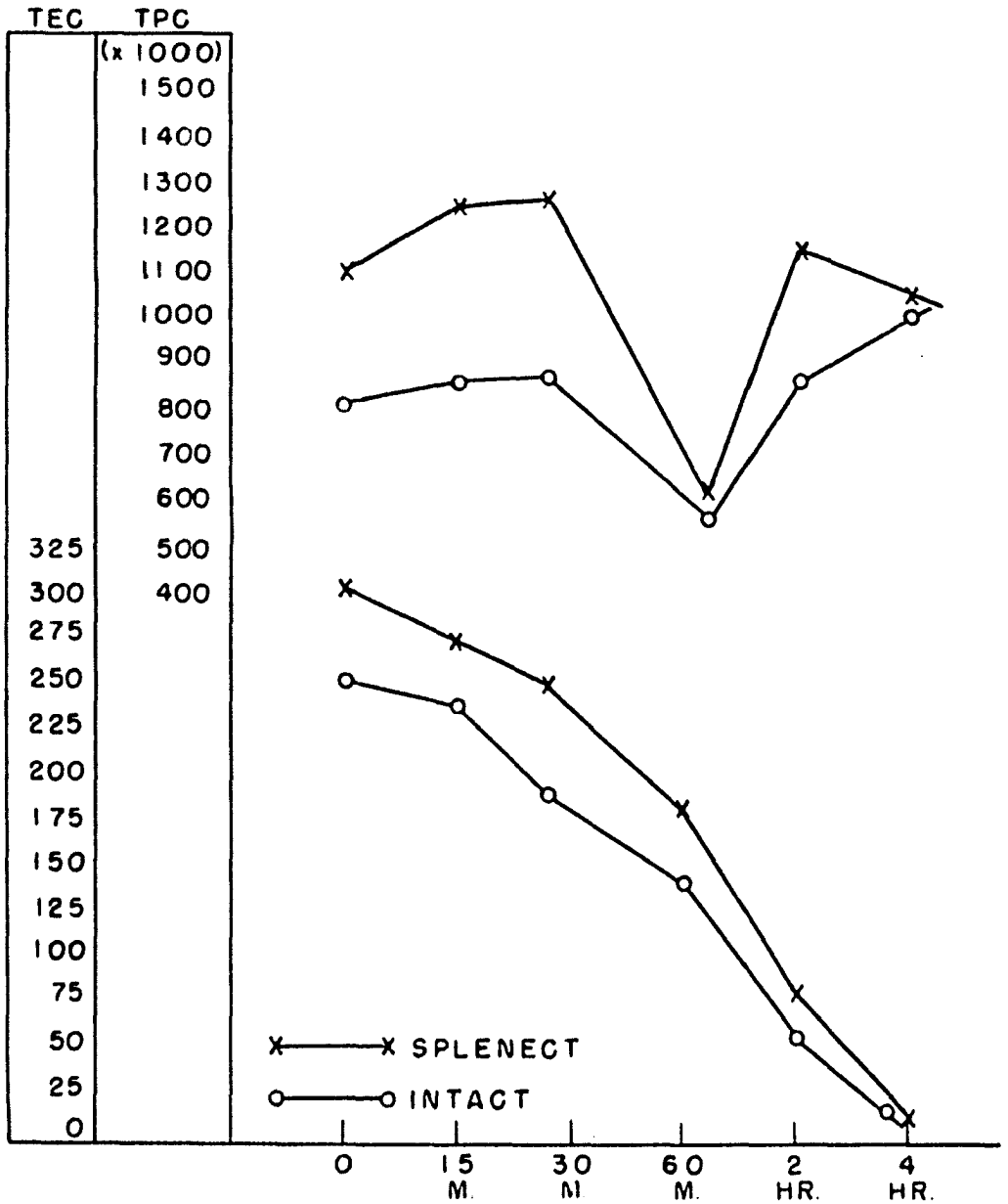

FIGURE 5. EFFECT OF CORTISONE ACETATE ON PLATELET AND EOSINOPHIL COUNTS OF SPLENECTOMIZED AND INTACT WHITE RATS.

F. Long-term Cortisone Acetate Experiment

1. Procedure

Twenty-seven healthy, two-month-old albino rats, weighing 270 ± 20 grams were used in this experiment. The design of the experiment was similar to the long term ACTH investigation. This time the objective was to find the effect of daily intraperitoneal injections of 2.5 mg. of cortisone acetate per 100 grams of body weight upon eosinophil, platelet and megakaryocyte counts of splenectomized and intact white rats over a period of 12 days.

After base counts of eosinophils, platelets and megakaryocytes had been made, 13 animals were splenectomized and 14 were left intact as control animals. 0-hour was 8:00 a.m. the first day of the experiment when the first injection was given and samples were taken for the 0-hour counts. Ten hours post 0-hour, counts were again made which were recorded as first day values. After the first day, the animals were injected between 8:00 and 10:00 a.m. and counts were made between 1:00 and 3:00 p.m. the same day. In other words, all count figures are afternoon results as in the ACTH long term experiment. This regularity was maintained throughout to obviate diurnal variation on the blood elements under investigation. In this experiment it became necessary to count alternate groups on alternate days after the seventh day. Irrespective of this, all animals received their daily dosage of cortisone within the 12-day experimental regimen. After 12 days of cortisone administration and sampling, the counting was extended to the eighteenth day to see if blood values would taper off after the exogenous hormone was withdrawn. Regular diet was maintained and no significant weight changes were noted within the experimental period.

2. Results

a. The splenectomized group. The base mean counts on this group were 124 eosinophils and 600,000 platelets per cu. mm., and 44 marrow megakaryocytes per 100 low power fields. At 0-hour, the mean eosinophil count was 165 per cu. mm. and the mean platelet count was 924,000 per cu. mm. The first day showed an eosinophil mean of 60 per cu. mm., a drop of 60 per cent, and a platelet mean of 1,040,000 per cu. mm., a gain of 12 per cent above 0-hour. On the second, third, and fourth days the platelets rose steadily: 1,213,000; 1,380,000; 1,571,000; or a 31, 48, and 70 per cent increase, respectively, above 0-hour. On the second day, the eosinophils fell to 46 per cu. mm.; the third day they were up a little to 61 per cu. mm., and on the fourth day they jumped to 121 per cu. mm. On the fifth day, the eosinophil mean was back down to 55 per cu. mm., or 67 per cent below 0-hour. The platelet mean was also down, slightly, to 1,472,000 per cu. mm., or 59 per cent above the 0-hour mean. On the seventh day the eosinophils had dropped to 21 per cu. mm., or 87 per cent below 0-hour mean; and the platelets had leveled off at 1,345,000 per cu. mm., now 44 per cent above 0-hour. On the ninth day the eosinophils dropped still more, to a mean of 3 cells per cu. mm., or 98 per cent below 0-hour. The ninth day platelet mean was 1,540,000 per cu. mm., or 66 per cent above 0-hour.

On the eleventh day the animals were in eosinopenia with only one cell per cu. mm., a 99 per cent decrease from 0-hour. Platelet values changed only slightly, 67 per cent above 0-hour with a mean of 1,554,000 per cu. mm. On the thirteenth day complete eosinopenia was present in the animals

with zero eosinophils per cu. mm. The platelets were still 41 per cent above the 0-hour level with a mean of 1,310,000 per cu. mm. On the sixteenth day, four days after discontinuation of the cortisone injections, the eosinophils had risen to 30 per cu. mm., and the platelet mean was 1,537,000 per cu. mm. On the eighteenth day, the eosinophils continued their rise to a mean of 61 cells per cu. mm., and the platelet mean dropped off slightly but was still 42 per cent above 0-hour with a mean of 1,317,000 per cu. mm.

The post-experimental megakaryocyte count was 71 cells per 100 low power fields, or an increase over the pre-experimental mean of 61 per cent.

b. The intact group. Basic means on this group were 118 eosinophils per cu. mm. and 641,000 platelets per cu. mm. The pre-experimental megakaryocyte mean was 44 cells per 100 low power fields. At 0-hour the eosinophil mean was 138 per cu. mm., and the platelet mean was 578,000 per cu. mm. The first day's count revealed an eosinophil decrease of 71 per cent, or a mean of 40 cells per cu. mm. The platelet mean advanced slightly the first day to 708,000 per cu. mm., an 11 per cent increase. On the second day the eosinophils dropped significantly and the platelets rose significantly; the eosinophils now showed 26 cells per cu. mm.; and the platelets 1,112,000 per cu. mm. On the third day the eosinophil mean climbed slightly to 83 per cu. mm., and the platelets continued their increase to 1,243,000 per cu. mm. This platelet mean was 111 per cent above 0-hour level.

On the fourth day, the eosinophils started dropping again, to 46 per cu. mm.; whereas the platelets were still climbing, now showing a 131 per

cent increase above 0-hour with a mean count of 1,335,000 per cu. mm. On the sixth day the eosinophils continued their downward trend that was to be maintained until the twelfth day, terminating in complete eosinopenia. It was shown as follows: sixth day, 16 per cu. mm.; eighth day, 10 per cu. mm.; tenth day, zero cells per cu. mm.; and twelfth day, zero cells per cu. mm. The blood platelets continued with significantly high values from the sixth to the twelfth days, inclusive, showing 148, 140, 147, and 153 per cent increases above 0-hour means on those respective days. On the fourteenth day, two days after cortisone treatment had been withdrawn, the eosinophils rose to 10 cells per cu. mm., and the platelets began dropping. The platelet mean on the fourteenth day was 995,000 per cu. mm. On the sixteenth day the eosinophils were continuing their increase, and the platelets continued their decrease. There now were 33 eosinophils per cu. mm., and 898,000 platelets per cu. mm. On the eighteenth day the eosinophils were back up to 55 cells per cu. mm. but still 60 per cent below the 0-hour mean. There were 835,000 platelets per cu. mm., still 44 per cent above the 0-hour mean.

The post-experimental megakaryocyte count revealed a value of 99 cells per 100 low power fields, an increase of 118 per cent over the pre-experimental mean.

The results are tabulated in Tables 13, 14, 15, and 16 and are graphically portrayed in Figure 6.

Table 13. Long Term[1] Cortisone Acetate[2] Effect upon Eosinophil[3] Co

Animal No. and group	Base Pre.Op.	0-hour	1-day	2-day	3-day	4-day	5-day	7-day	9-d
1 - A	88	100	25	6	38	69	38	6	6
3 - A	175	156	38	0	75	88	25	0	(
4 - A	112	88	31	13	75	144	44	19	(
0 - A	119	188	63	0	138	125	31	0	(
1 - B	81	194	50	63	63	150	50	25	(
3 - B	138	219	75	75	50	94	112	63	(
4 - B	100	181	100	112	75	175	75	-- Animal	1
5 - B	100	163	88	125	69	169	44	13	(
0 - B	156	206	106	106	94	112	31	6	(
1 - E	163	188	94	75	56	131	44	25	1
3 - E	63	181	63	25	19	144	50	19	(
4 - E	138	225	38	13	38	119	63	31	2
5 - E	138	150	25	0	25	81	75	0	(
0 - E	175	75	50	38	50	100	88	69	(
No. of animals-14									
Min.	63	75	25	0	19	81	25	0	
Max.	175	225	106	125	138	175	112	69	2
Mean	124	165	60	46	61	121	55	21	
% Change[6]		+33	-63	-72	-63	-26	-67	-87	-9

[1] Cortisone acetate administered daily, first to twelfth day inclusive.

[2] Dose 02.5 mg. cortisone acetate per 100 grams of body weight intraperitone

[3] Values are given in number of cells per cu. mm.

[4] Weight, 270 ± 20 grams

[5] Six days post splenectomy

[6] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change

Cortisone Acetate[2] Effect upon Eosinophil[3] Counts of the Splenectomized Rat[4].

2-day	3-day	4-day	5-day	7-day	9-day	11-day	13-day	16-day	18-day
6	38	69	38	6	6	0	0	13	25
0	75	88	25	0	0	0	0	44	44
13	75	144	44	19	0	0	0	25	31
0	138	125	31	0	0	0	0	6	38
63	63	150	50	25	0	0	0	50	75
75	50	94	112	63	0	0	0	56	63
112	75	175	75	-- Animal removed from experiment --					
125	69	169	44	13	6	0	0	44	63
106	94	112	31	6	0	0	0	63	88
75	56	131	44	25	13	6	0	31	75
25	19	144	50	19	0	0	0	13	81
13	38	119	63	31	25	13	0	13	56
0	25	81	75	0	0	0	0	6	81
38	50	100	88	69	0	0	0	25	69
0	19	81	25	0	0	0	0	6	25
125	138	175	112	69	25	13	0	63	88
46	61	121	55	21	3	1	0	30	61
-72	-63	-26	-67	-87	-98	-99	-100	-82	-63

ed daily, first to twelfth day inclusive.
ate per 100 grams of body weight intraperitoneal.
f cells per cu. mm.

nge

Table 14. Long Term[1] Cortisone Acetate[2] Effect on Platelet[3] and Megak
Rat[4].

Animal No. and group	Base Pre-Op.	0-hour[5]	1-day	2-day	3-day	4-day	5-day	7-day	9-day	11-day	13
1-A	520	800	1110	1000	1310	1400	1070	1190	1640	1140	1
3-A	600	870	900	1210	1510	1610	1790	1420	2180	1050	1
4-A	500	840	1200	1300	1670	1320	1200	720	1700	1900	1
0-A	510	970	1000	1320	1600	1620	1310	1400	1230	1470	1
1-B	640	1050	1260	1200	1400	1700	1780	1300	1300	1480	1
3-B	560	800	830	1190	1320	1410	1410	1410	1600	1840	1
4-B	650	1200	1000	1320	1410	1820	1240	-- Animal removed fro			
5-B	600	960	1030	1100	1200	1860	1970	1490	1170	1390	
0-B	610	860	1050	1210	1340	2430	1790	1530	1520	1520	
1-E	530	900	1000	1300	1240	1300	1600	1460	1200	1320	
3-E	760	890	980	1200	1300	1400	1450	1520	1340	1820	
4-E	510	1120	1120	1140	1430	1500	1200	1360	2000	1970	
5-E	510	940	1140	1320	1200	1430	1500	1490	1520	1590	
0-E	710	840	940	1180	1400	1310	1300	1200	1600	1720	
No. of animals-13											
Min.	500	800	830	1000	1200	1300	1070	720	1170	1050	
Max.	760	1200	1200	1320	1670	1860	1970	1530	2180	1970	
Mean	600	924	1040	1213	1380	1571	1472	1345	1540	1554	
% Change[6]		+54	+12	+31	+48	+70	+59	+44	+66	+67	

[1]Cortisone acetate administered daily, first to twelfth day inclusive.
[2]Dose, 02.5 mg. cortisone acetate per 100 grams body weight, intraperitoneal.
[3]Multiply platelet values by 1000 for number per cu. mm.
[4]Weight, 270 ± 20 grams.
[5]Six days post splenectomy.
[6]$\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change.

isone Acetate[2] Effect on Platelet[3] and Megakaryocyte Counts of Splenectomised

3-day	4-day	5-day	7-day	9-day	11-day	13-day	16-day	18-day	Megakaryocytes Pre-Exp. /100 L.P.F.	Megakaryocytes Post-Exp. /100 L.P.F.
1310	1400	1070	1190	1640	1140	1580	1200	1120	62	68
1510	1610	1790	1420	2180	1050	1460	1000	1000	26	56
1670	1320	1200	720	1700	1900	1480	1970	1500	34	86
1600	1620	1310	1400	1230	1470	1000	1500	1200	41	89
1400	1700	1780	1300	1300	1480	1370	1440	1140	34	66
1320	1410	1410	1410	1600	1840	1180	1800	1400	57	47
1410	1820	1240	-- Animal removed from experiment --							
1200	1860	1970	1490	1170	1390	1210	1400	1500	42	86
1340	2430	1790	1530	1520	1520	1050	1480	1240	63	63
1240	1300	1600	1460	1200	1320	1260	1210	1100	26	58
1300	1400	1450	1520	1340	1820	1480	1800	1600	47	64
1430	1500	1200	1360	2000	1970	1630	1920	1400	51	71
1200	1430	1500	1490	1520	1590	1100	1600	1320	43	76
1400	1310	1300	1200	1600	1720	1240	1680	1600	32	88
1200	1300	1070	720	1170	1050	1050	1000	1000	26	56
1670	1860	1970	1530	2180	1970	1580	1970	1600	63	88
1380	1571	1472	1345	1540	1554	1310	1537	1317	44	71
.48	.70	.59	.44	.66	.67	.41	.66	.42		.61

ly, first to twelfth day inclusive.
per 100 grams body weight, intraperitoneal.
for number per cu. mm.

Table 15. Long Term[1] Cortisone Acetate[2] Effect on Eosinophil[3] Co

Animal No. and group	Base Pre-Op.	0-hour[5]	1-day	2-day	3-day	4-day	6-day	8-da
1 - C	125	206	50	38	100	38	13	0
3 - C	163	200	19	63	112	44	0	13
4 - C	131	88	44	19	31	50	0	0
5 - C	150	169	13	13	125	31	0	0
0 - C	119	94	56	0	38	0	0	13
1 - D	112	169	25	38	88	19	0	19
3 - D	94	112	50	63	69	25	25	44
4 - D	100	138	75	--	Animal removed from experiment			
5 - D	63	100	25	0	106	31	19	0
0 - D	131	94	44	6	25	44	63	0
1 - F	169	75	31	25	75	56	0	0
3 - F	75	125	13	0	81	31	38	25
4 - F	94	150	63	38	63	69	0	0
5 - F	156	163	50	50	112	119	25	19
0 - F	88	188	38	13	138	94	50	31
No. of animals-15								
Min.	63	75	13	0	25	0	0	0
Max.	169	206	75	138	138	119	63	44
Mean	118	138	40	83	83	46	16	10
% Change[6]		+16	-71	-81	-31	-62	-88	-92

[1] Cortisone acetate administered daily, first to twelfth day inclusiv

[2] Dose, 02.5 mg. cortisone acetate per 100 grams body weight, intrape

[3] Eosinophil values are number per cu. mm.

[4] Weight 270 ± 20 grams.

[5] Six days post-splenectomy.

[6] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change.

Cortisone Acetate[2] Effect on Eosinophil[3] Counts of the Intact Rat[4].

-day	2-day	3-day	4-day	6-day	8-day	10-day	12-day	14-day	16-day	18-day
50	38	100	38	13	0	0	0	0	25	44
19	63	112	44	0	13	13	0	13	25	31
44	19	31	50	0	0	0	0	25	38	50
13	13	125	31	0	0	6	0	13	13	19
56	0	38	0	0	13	0	0	0	6	25
25	38	88	19	0	19	0	0	6	31	44
50	63	69	25	25	44	6	0	13	44	63
75	-- Animal removed from experiment --									
25	0	106	31	19	0	0	0	13	31	38
44	6	25	44	63	0	0	0	19	25	50
31	25	75	56	0	0	19	0	6	44	75
13	0	81	31	38	25	25	0	13	38	88
63	38	63	69	0	0	0	0	0	13	31
50	50	112	119	25	19	0	0	25	56	94
38	13	138	94	50	31	0	0	19	81	125
13	0	25	0	0	0	0	0	0	6	19
75	138	138	119	63	44	25	0	25	56	125
40	83	83	46	16	10	5	0	10	33	55
-71	-81	-31	-62	-88	-92	-95	-100	-92	-76	-60

stered daily, first to twelfth day inclusive.

acetate per 100 grams body weight, intraperitoneal.

mber per cu. mm.

y.

% change.

Table 16. Long Term[1] Cortisone Acetate[2] Effect on Platelet[3] and Megakaryocy

Animal No. and group	Base Pre-Op.	0-hour[5]	1-day	2-day	3-day	4-day	6-day	8-day	10-day	12-day	14-d
1 - C	540	570	970	1400	1420	1460	1030	1250	1280	1500	750
3 - C	720	580	880	1100	1120	1100	1730	1320	1340	1300	980
4 - C	500	640	800	1140	1200	1500	1420	1200	1220	1120	1130
5 - C	610	760	600	1170	1240	1330	1150	1000	1110	1340	870
0 - C	730	670	660	1050	1400	1610	1240	1500	1600	1900	1100
1 - D	760	610	790	1100	1600	1640	1500	1660	1680	1500	1100
3 - D	720	500	530	1000	1100	1300	1660	1740	1700	1770	700
4 - D	640	590	690	-- Animal removed from experiment --							
5 - D	670	500	810	1140	1050	1200	1970	1170	1200	1020	1130
0 - D	600	520	860	1120	1200	1250	1370	1480	1500	1330	1150
1 - F	670	450	640	1000	1490	1600	1400	1390	1400	1560	900
3 - F	600	540	510	1050	1400	1200	1320	1720	1670	1730	1200
4 - F	600	660	720	1120	1030	1000	1560	1640	1800	1840	860
5 - F	650	510	660	1160	1100	1210	1280	1100	1000	1030	940
0- F	760	580	500	1030	1060	1300	1500	1260	1420	1570	1120
No. of animals-14											
Min.	500	450	500	1000	1100	1000	1030	1000	1000	1020	700
Max.	760	760	970	1400	1490	1640	1970	1740	1800	1900	1200
Mean	641	578	708	1112	1243	1335	1437	1387	1424	1465	995
% Change[6]		+9	+11	+91	+111	+131	+148	+140	+147	+153	+73

[1] Cortisone acetate administered daily, first to twelfth day inclusive.

[2] Dose, 02.5 mg. cortisone acetate per 100 grams body weight, intraperitoneal.

[3] Multiply platelet values by 1000 for number per cu. mm.

[4] Weight, 270 ± 20 grams.

[5] Ten to fifteen days post-splenectomy.

[6] $\frac{\text{0-hour - 1-day, etc.}}{\text{0-hour}}$ = % change.

Acetate[2] Effect on Platelet[3] and Megakaryocyte Counts of the Intact Rat[4].

y	4-day	6-day	8-day	10-day	12-day	14-day	16-day	18-day	Megakaryocytes Pre-Exp. /100 L.P.F.	Megakaryocytes Post-Exp. /100 L.P.F.
0	1460	1030	1250	1280	1500	750	720	600	24	98
0	1100	1730	1320	1340	1300	980	900	800	31	105
0	1500	1420	1200	1220	1120	1130	1020	930	46	94
0	1330	1150	1000	1110	1340	870	850	820	52	92
0	1610	1240	1500	1600	1900	1100	940	880	29	96
0	1640	1500	1660	1680	1500	1100	900	760	38	110
0	1300	1660	1740	1700	1770	700	730	700	43	100
1	removed from experiment --									
0	1200	1970	1170	1200	1020	1130	950	900	52	95
0	1250	1370	1480	1500	1330	1150	1040	920	66	105
0	1600	1400	1390	1400	1560	900	870	800	38	112
0	1200	1320	1720	1670	1730	1200	1120	890	44	90
0	1000	1560	1640	1800	1840	860	840	880	58	79
0	1210	1280	1100	1000	1030	940	900	920	69	80
0	1300	1500	1260	1420	1570	1120	1000	890	27	86
0	1000	1030	1000	1000	1020	700	720	600	24	79
0	1640	1970	1740	1800	1900	1200	1120	930	69	112
3	1335	1437	1387	1424	1465	995	898	835	44	96
1	±131	±148	±140	±147	±153	±73	±55	±44		±118

, first to twelfth day inclusive.
100 grams body weight, intraperitoneal.
number per cu. mm.

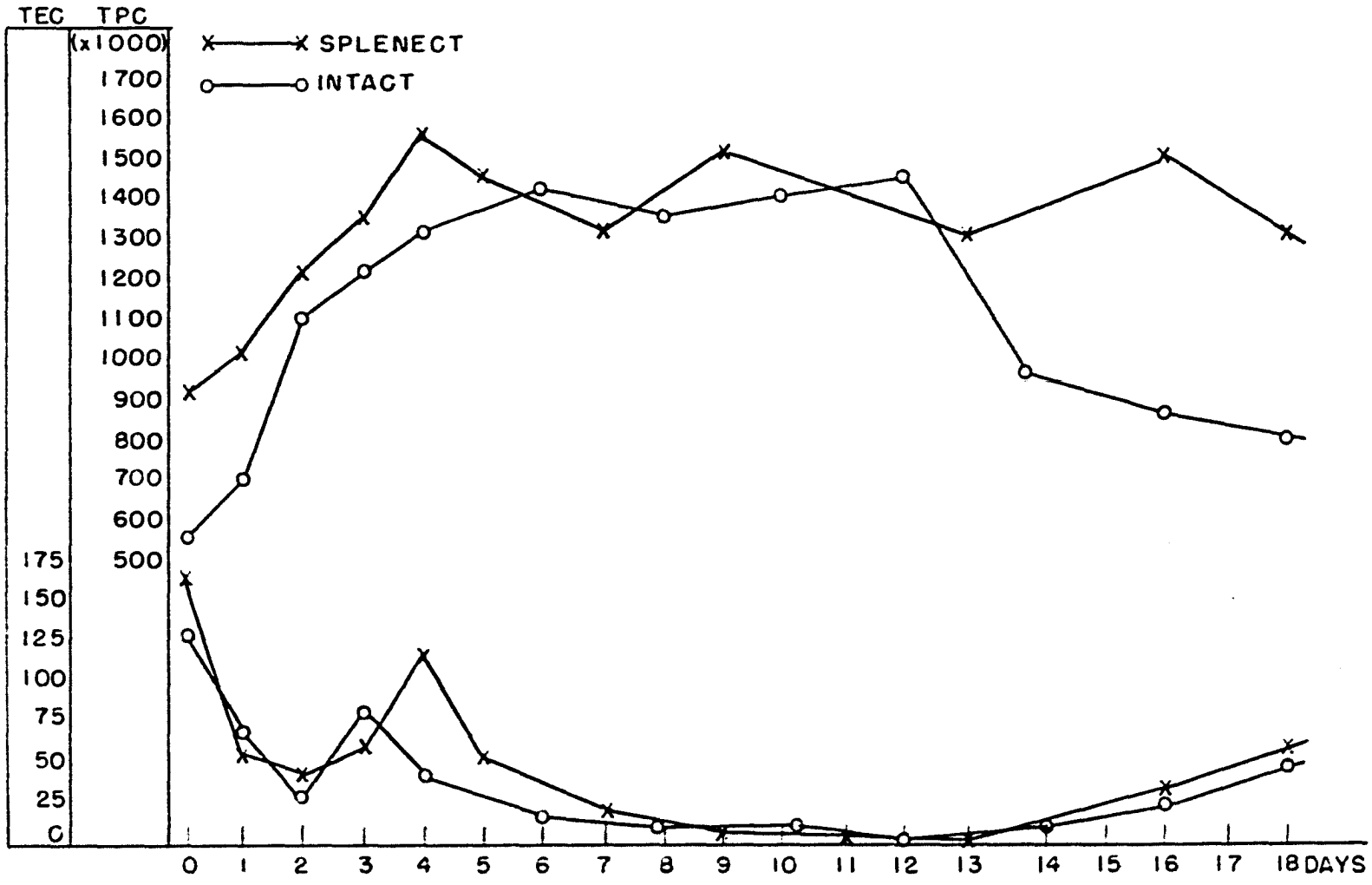

FIGURE 6. EFFECT OF DAILY CORTISONE ACETATE ON PLATELET AND EOSINOPHIL COUNTS OF SPLENECTOMIZED AND INTACT WHITE RATS.

VI. DISCUSSION

A. Saline Pilot Experiment

Inasmuch as this investigation was instigated to find what effect adrenal hormones had upon blood platelets, eosinophils and megakaryocytes of the white rat, it was thought necessary to rule out all side effects upon the experimental animals that might occur from handling, anesthetizing and injecting.

The saline group which was made up of both splenectomized and intact animals showed no significant platelet changes throughout the length of this experiment. The platelet means, as seen in Figure 1, plotted nearly a straight line. The eosinophils, however, showed significant decreases in both groups at the two and four hour periods. It should be pointed out that the tendency toward an eosinopenia was not demonstrated until four hours after injection of saline, whereas, as will be noted in subsequent experiments, pronounced eosinopenia was evident within sixty minutes after treatment.

As a result of this experiment the investigator was satisfied that handling, injecting and blood sampling for counts would not produce a platelet effect of any significance in the future experiments. It was interesting to note, however, the sensitivity of the eosinophils even to such a small amount of mechanical stress as was applied in this pilot experiment; in contrast, platelet counts were unaffected. This substantiated Thorn et al. (1953) who stated that the eosinophil count is the most sensitive stress index that we have at the present time.

B. Epinephrine Experiment

It has been well established that epinephrine is discharged in most situations of acute stress and must, according to Sayers (1949), play an important role in the discharge of ACTH in all these situations. Exactly what the mechanism of epinephrine action is, with reference to the pituitary-adrenocortical system, has not yet been made clear, but a brief discussion of recent findings and postulates will be presented in view of the results of the present investigations.

Gellhorn (1949), using cortical vitamin C depletion as an index of cortical activity, demonstrated that animals exposed to cold stress showed a marked decrease in adrenal ascorbic acid. However, if the animals were anesthetized with sodium pentobarbital and then put into the cold room, no vitamin C depletion occurred. Thorn (1949) found that during major operations on human patients, under ether, the eosinophil count fell to zero within four hours; but if the operation was performed under spinal anesthesia there was no decrease in eosinophils until after the anesthesia had worn off. On the basis of this fact, Thorn (1949) suggested that epinephrine stimulates the anterior hypothalamus and, as a result, a humorally transmitted substance is released which, in turn, acts upon the anterior pituitary to induce ACTH secretion. This humoral transmittor, in Thorn's opinion, may also be produced by stimuli from higher centers; so apparently epinephrine is not the only ACTH stimulator. Thorn (1949) explained the sodium pentobarbital action as taking place in one of two ways: either it cut down the amount of epinephrine released by the adrenal medulla, or the pentobarbital had a local effect upon the

hypothalamus, inhibiting its response to epinephrine.

Sayers (1949), Long (1952) and Thorn et al. (1953) are all in agreement that the eosinophil is, at present, the most sensitive index known as a measure for cortical hormone secretion.

It was not the object of the present investigation to attempt a clarification of the intricate action of epinephrine but rather to elucidate, if possible, the effect of the various stressor agents upon the blood platelets, eosinophils and marrow megakaryocytes of the splenectomized and intact white rat.

A comparison of the mean platelet increases, after epinephrine, as shown in Tables 2, 3, and 4 and Figure 2, of the splenectomized, sham-splenectomized and intact rats, revealed that the sham-splenectomized and intact rats had a far greater increase in mean platelet values than did the splenectomized group. The results following epinephrine showed a 65 per cent mean platelet increase at the 15 minute period and a 43 per cent mean platelet increase at the 30 minute period for the intact group. The sham-splenectomized group showed mean platelet increases of 69 per cent and 66 per cent within the same periods of time; whereas, the splenectomized animals showed only 21 per cent and 24 per cent increases in the same periods of time. As a $\pm$ 20 per cent change equilibrates with a standard deviation of 68,000 in normal unoperated rats (Ottis, 1951), changes of the magnitude of 65, 43, 69, and 66 per cent show a high degree of significance. Such a significant contrast in platelet values between splenectomized and non-splenectomized animals would seem to implicate the spleen as a control mechanism of the circulating blood platelets for the animal under stress. These results are comparable, in part, to the findings of Ungar (1945) in

clotting time tests on splenectomized and non-splenectomized guinea pigs, inasmuch as he reported a significant difference in the clotting time for splenectomized animals as compared with the clotting time for intact animals. Ungar (1945) interpreted his results to the effect that splenectomy had protected his animals against the stress response of the pituitary-adrenocortical axis. A complete comparison, however, between Ungar's work and the present investigation is impossible because Ungar (1945) did not support the clotting time tests with concurrent platelet and eosinophil counts. Furthermore, without concurrent eosinophil counts, blood cholesterol level tests, or cortical vitamin C depletion tests, Ungar (1945) could only postulate that his animals were under stress.

Regarding the controversy as to whether epinephrine is a brief acting, non-specific stressing agent or a "trigger" for the pituitary-adrenocortical axis, reference to Figure 2 shows all three groups approaching normal 0-hour levels for platelet counts at the 60 minute period. This fact would seem to support the contention that the action of epinephrine is of an emergency nature and of short duration in its effect. Further investigation, however, is necessary to clarify this point for, if the platelet values were still dropping at the two and three hour periods after epinephrine, it would indicate that ACTH had then taken over. This interpretation is made in the light of the ACTH short-term experiment, that will be discussed subsequently, in which platelet values dropped sharply after a single physiological dose of ACTH.

A secondary point of interest is to be found in the fact that the mean platelet values of the splenectomized group rose from a 0-hour mean of 1,447,000 per cu. mm. to a 30-minute mean of 1,940,000 platelets per

cu. mm. after epinephrine. This fact is somewhat contrary to the information in several modern physiology textbooks which state that platelet increases, post-epinephrine, are due to splenic contraction (Best and Taylor, 1945; Evans, 1952; Zoethout and Tuttle, 1952). It would seem that the theory of "splenic contraction", which dates back to the original work of Cannon and Gray (1914), is somewhat passe in the light of the present investigation. The spleen is not necessary for moderate increases in platelet values, post-epinephrine, in the white rat.

The eosinophil means, post-epinephrine, showed significant drops at both the 30 minute period and the 60 minute period in all three experimental groups. There was, however, no significant difference among the groups in the mean percentage decreases as they showed a -81 per cent, a -80 per cent, and a -78 per cent decrease in mean eosinophil values at 60 minutes for the splenectomized, sham-splenectomized and intact groups, respectively. Since a significant decrease in the eosinophil count is considered a good indication of hyperadrenocortical activity (Thorn et al., 1953), the above data would indicate that all three groups were being stressed equally by epinephrine in this experiment. This being the case, the findings of this experiment would disprove Ungar's (1945) postulate that splenectomy protects the animal against pituitary-adrenocortical response.

C. Short-term ACTH Experiment

As a consequence of the results obtained in the epinephrine experiment, the question arose concerning the effect a single physiological dose of ACTH would have upon the blood platelets and the marrow megakaryocytes of the splenectomized and intact white rat. Reference to the literature was

of little help in this matter as it revealed only a controversy of reports and interpretations concerning ACTH effects upon blood platelets. No controlled experiment had been done in which adrenocortical stress had been measured concurrently with the blood platelet counts. For this reason the short-term ACTH experiment was instituted to find the effect of a single injection of ACTH on the blood platelets, eosinophils and marrow megakaryocytes of the splenectomized and intact white rat over a period of ten hours following injection.

Reference to the results of the ACTH short-term experiment revealed some very significant effects of ACTH as reflected by the 33 per cent, 48 per cent, and 47 per cent decreases in the platelet means at the 1, 2, and 3 hour periods, respectively, after ACTH injection. The splenectomized animals were also the first to indicate a developing eosinopenia after ACTH with a decrease of 48 per cent in the eosinophil means at the two hour period post-ACTH. The splenectomized group started to recoup their platelet losses at the 4 hour period and at the 10 hour period were within 27 per cent of their 0-hour level. Adrenocortical stress, however, remained constant throughout the 10 hour period as was revealed by the mean decrease of 93 per cent in the splenectomized mean at the end of the experiment.

The intact group was slower than the splenectomized group to develop a thrombocytopenia. The intact animals showed a mean platelet decrease of 44 per cent at the three hour period, post-ACTH injection; but these values tapered off to a 41 per cent decrease at the four hour period, followed by a 31 per cent decrease at ten hours post-ACTH injection. Eosinopenia developed more slowly, also, in the intact group; but by ten

hours they too were in complete eosinopenia with a 93 per cent mean decrease in eosinophils from the 0-hour mean.

Both splenectomized and intact animals showed an increase in marrow megakaryocytes at the end of the experiment as compared to their pre-experimental values. There were, however, no significant differences between groups as the splenectomized mean increase was 62 per cent and the intact mean increase was 68 per cent above the pre-experimental values.

These data support, in part, reports of the effects of ACTH upon clotting time tests upon patients undergoing ACTH therapy (Jacques, 1954). The clotting time of these patients was lengthened at the 3-4 hour period post -ACTH injection but returned to normal in 24 hours. This was followed, within the next 3-4 days, by a shortening of the clotting time in the same patients. Stefanini and Rosenthal (1950) have also reported initial hemorrhagic manifestations in two patients on ACTH therapy.

D. Long-term ACTH Experiment

After the results of the short-term ACTH experiment had been analyzed, it was apparent that further information was needed to ascertain the long-term effect of ACTH upon blood platelets. Because of the significant depressive action of ACTH upon platelets in both the splenectomized and intact rats in the short-term experiment, the question arose as to whether daily injections of ACTh over a two-week period would continue to depress platelet numbers. In an attempt to find the answer to this question the long-term ACTH experiment was undertaken.

The first day's data of the long-term ACTH experiment were consistent

with the results of the short-term experiment. The results indicated that the platelet means of both splenectomized and intact animals dropped, the mean for the splenectomized group to a greater degree. This platelet decrease was followed by a return to 0-hour levels on the second day of ACTH injections. On the third and succeeding days platelet numbers increased with slight variations. The greatest mean increases were reached on the ninth and eleventh days for the splenectomized and intact groups, respectively. The former showed a mean platelet increase of 52 per cent over the 0-hour level; the latter, a 134 per cent mean increase.

During daily injections of ACTH there was a significant difference in the platelet response of the splenectomized animals as compared with that of the intact animals. As was noted in the epinephrine experiment, the intact animals showed much greater platelet increases than did the splenectomized animals while both groups were undergoing epinephrine stress. In the ACTH long-term experiment, when both groups were receiving the same daily dosage of ACTH, they both showed significant increases in platelet numbers; however, the mean increase of the intact group was significantly higher than the mean increase of the splenectomized group. Although there was a significant difference between the platelet values of the two groups, the eosinophil response was constant. From the second day on, and through the fourteenth day, when daily injections of ACTH were discontinued, both splenectomized and intact animals were in almost complete eosinopenia. These eosinophil results indicated that both groups were under almost equal adrenocortical stress.

The marked difference in percentage increase of the platelet mean of the intact group, under ACTH, over those of the splenectomized group was

quite apparent in this experiment. This is verified by the marked increase of 134 per cent on the ninth and eleventh days for the intact group; whereas the splenectomized animals showed increases but of a lesser magnitude, namely a maximum of 52 per cent on the same days. The platelet values of the intact group, treated with ACTH, behaved much like those of splenectomized rats without ACTH stimulation. Splenectomy is singularly effective in raising the platelet values in the mammal. It may be interpreted, therefore, from this experiment that ACTH is very effective in bringing about an increase in platelet numbers in the intact animal. That these significant platelet increases were not present in the splenectomized animal, under ACTH, leads one to conclude that splenectomy, in some way, has modified the animal's ability to respond to this adrenocortical stimulating hormone. These findings were of special significance when it was noted that both animal groups were under equal adrenocortical stress as was substantially affirmed by their eosinopenic condition throughout the platelet sampling period of this experiment.

A comparison of the post-experimental megakaryocyte counts with the pre-experimental megakaryocyte counts revealed significant increases for both the splenectomized and intact rats. Again, a significant difference between the two groups was noted. The splenectomized animals showed a mean increase of 92 per cent; while the intact animals showed a mean increase of 153 per cent of marrow megakaryocytes post-experimentally. These data would support in full the platelet response described above.

In reviewing the data of the short-term and long-term ACTH experiments, it is to be noted that platelet numbers were depressed during the first ten hours of both experiments, but that in the long-term experiment this decrease

was followed by a return to 0-hour levels and later by significant increases for both intact and splenectomized animals. These findings would support, in part, Jacques' report of 1954 on clotting time test variations of patients undergoing ACTH therapy.

E. Short-term Cortisone Acetate Experiment

As was stated in the discussion of the ACTH experiments the "triggering" of adrenocortical secretion has been amply demonstrated and documented. It was a natural sequence of events, then, to follow the ACTH study with one in which the direct administration of one of the active corticoids was carried out and its effects noted on the blood platelets, eosinophils and marrow megakaryocytes of the splenectomized and intact white rat. It was reasoned that the cortisone dosage must be sufficient to cause a precipitous eosinopenia. Then, with the experimental animals under this simulated stress condition, platelet counts would be taken throughout a four hour period and any or all physiological variations noted and tabulated.

Cortisone is one of the series of crystalline substances isolated from extracts of the adrenal cortex by Dr. E. C. Kendall of the Mayo Foundation. As was stated in the Review of Literature, this substance was originally named Compound E and was isolated from beef adrenal glands. Cortisone acetate, Merck, was first synthesized through the combined efforts of Dr. L. H. Sarett of Merck and Company and Dr. E. C. Kendall of the Mayo Foundation. Thus, the compound available in quantities for animal experimentation, and, therefore, the one used in both the short-term ahd long-term cortisone experiments in this investigation, was this synthetic product, 11-Dehydro-17 Hydroxy Corticosterone-21-Acetate. Loeb reported

in 1949 that the steroids most active in protecting the animal against stress were primarily those oxygenated at C-11.

The results of the short-term cortisone experiment revealed certain similarities to the short-term ACTH experiment. It was noted that platelet numbers were depressed in both of these experiments, more so in the ACTH experiment than in the cortisone experiment, however. In the cortisone short-term experiment the platelet means for both the splenectomized and intact animals dropped precipitously at the sixty minute period. The most significant decrease was experienced by the splenectomized group whose platelet mean decreased 42 per cent at sixty minutes post-cortisone injection. At the two hour period, however, platelet means for both groups rose again to approximately 0-hour levels and remained there with slight variations to the end of the experimental period.

The eosinophil means showed almost a straight line drop from 0-hour to the four hour period of the experiment for both splenectomized and intact animals. At the four hour period both groups were in complete eosinopenia. This being the case, it was safe to say that both groups were under the same amount of simulated adrenocortical stress. Furthermore, the blood platelets also were under the same stress situation and responded as has been described. The findings of this experiment, therefore, were not in agreement with the report from Adams (1949) which stated no significant changes in platelet numbers for either intact or splenectomized rats after injections of cortisone acetate.

F. Long-term Cortisone Acetate Experiment

After studying the results of the short-term cortisone acetate experiment, it became apparent that still more information was needed concerning the effect of cortisone acetate upon the blood platelets, eosinophils and marrow megakaryocytes of the splenectomized and intact white rat. The question arose as to whether, upon continued administration of cortisone, the platelet values would continue to increase or would decline into a second depression period. The literature was conflicting on this point; so, a long-term experiment was initiated in which splenectomized and intact animals received cortisone acetate daily for 12 days. Platelet and eosinophil counts were made at regular intervals throughout the 12-day period. Marrow megakaryocyte counts were made both pre-experimentally and post-experimentally as in the previous experiments.

An analysis of the data of the long-term cortisone experiment revealed that from the first day, through the fourth day, the platelet means of both groups rose steadily. On the fourth day the platelet mean for the splenectomized group was 70 per cent above the 0-hour mean and the platelet mean for the intact group was 131 per cent above their 0-hour mean. The platelet means of both groups held this high level with very little variation for the 12 days of the experiment.

That both groups showed significant platelet increases under cortisone administration was obvious from these data; however, there was also a significant difference between the two groups. The platelet mean of the intact group rose to 148 per cent above 0-hour on the sixth day, but the platelet mean of the splenectomized group never rose above its early 70

per cent increase of the fourth day. Once again it was noted that the intact animals responded more significantly to hormonally induced stress than did the splenectomized rats in regard to mean platelet numbers.

Upon examining the stress index, the eosinophil counts, it was noted that the animals of both groups responded immediately to the cortisone acetate injections with significant decreases in eosinophil numbers. Sufficient stress, however, was not immediate as indicated by a temporary rise in the eosinophils of both groups on the third and fourth days. This increase was short lived as was seen by the precipitous drop in eosinophil means on the fifth and sixth days which carried the eosinophil means to eosinopenic levels from which they never recovered during the length of the experiment. In fact, the animals were still in a state of eosinopenia two days after the cortisone injections had been discontinued. These eosinophil data indicated that both groups of animals were under considerable induced stress and were responding equally as far as the eosinophil index was concerned. It was of interest to note that this eosinophil response is consistent with that of the ACTH long-term experiment.

The megakaryocyte data also support the platelet response of both the splenectomized and intact animals in this experiment. The splenectomized animals showed a megakaryocyte mean increase of 61 per cent post-experimentally; whereas, the intact group showed an increase of 118 per cent over their pre-experimental mean, nearly twice the increase of the splenectomized rats.

A careful examination of the data of the short-term and long-term ACTH and cortisone experiments revealed the fact that splenectomy seemed to cause little or no difference in platelet responses during the first 12

hours of any of these four experiments. In both short-term experiments, and during the first 12 hours of the long-term experiments, the response was significant but similar for both splenectomized and intact animals. Beyond the 12 hour period in the long-term experiments, the responses were significant but dissimilar in that the platelet responses of the intact animals were significantly higher than those of the splenectomized animals.

There are two possible explanations to the platelet responses of the splenectomized and intact animals undergoing ACTH and cortisone acetate injections. Either the removal of the spleen modified or protected the animal against further accesses in platelet numbers or a "physiological limit" had been reached in the splenectomized animals, beyond which no significant blood platelet increases were probable. It cannot be deduced from the results of these experiments that splenectomy protected the animal against pituitary-adrenocortical stress, as is suggested by Ungar (1945), for there is no question that the animals were under stress when the eosinophil index is examined. Rather, it should be proposed that splenectomy has altered, in some way, the animal's blood platelet response to adrenocortical stress.

VII. SUMMARY AND CONCLUSIONS

A. Review of Literature

A survey of pertinent literature concerning the morphology and physiology of mammalian blood platelets, from the original description by Donne in 1842 to 1950 had been prepared previously by this investigator and was brought up to date in the present study. The literature is fragmentary and contradictory as to the effect of adrenal and adrenocortical hormones on blood platelets and megakaryocytes. No study seems to have been made to find the effect of adrenal and adrenocortical hormones upon blood platelet values accompanied by a stress indicator such as the eosinophil count. The role of the spleen as to its regulatory effect upon platelets and megakaryocytes is not clear. It is proposed by some that the action of the spleen is hormonal in nature in connection with platelet delivery from the bone marrow.

B. Materials and Methods

One-hundred fifty-eight, two- to three-month old rats of the Holtzman-Rolfsmeyer strain were used in this investigation. A method for counting blood platelets, using siliconized pipettes to eliminate platelet fragmentation was used. An eosinophil diluent for rat blood and an eosinophil counting technique were described. A technique for obtaining bone marrow samples from the anesthetized rat was developed. A surgical technique for splenectomizing the rat, using an electric cautery, was described.

C. Experimental

1. Procedure and results of saline experiment

A preliminary saline injection experiment was run on four splenectomized and four intact rats in an effort to learn the effect of handling, anesthetizing and injecting upon platelet and eosinophil values of the splenectomized and intact rat.

Blood platelet counts of both intact and splenectomized rats, taken at 0-hour, fifteen minute, thirty minute, sixty minute, two hour and four hour periods showed no significant change throughout the period of observation. Eosinophil counts on intact and splenectomized rats showed no significant change until the two hour period, at which time they dropped sharply.

2. Procedure and results of epinephrine experiment

A short-term experiment was run on 20 splenectomized, 12 sham-splenectomized and six intact white rats to ascertain the effect of 0.25 ml. of epinephrine, per 100 grams of body weight, upon blood platelet, eosinophil and megakaryocyte values of these animals. Base platelet, eosinophil and megakaryocyte counts were taken before surgical intervention. The time of injection of epinephrine and the immediate withdrawl of the first blood sample were designated as the 0-hour when the first post-treatment count was made. Serial counts followed at fifteen, thirty, and sixty minutes post 0-hour. Post-experimental marrow counts were made three days after the experimental regimen had been completed.

A comparison of the mean platelet increases, after epinephrine, of

the splenectomized, sham-splenectomized and intact rats revealed that the sham and the intact rats had far greater increases in mean platelet values than did the splenectomized group. The platelet values for the three groups increased as much as 65, 69 and 24 per cent above their respective 0-hour levels for the intact, sham-splenectomized and splenectomized groups, respectively.

3. Procedure and results of the ACTH short-term experiment

Sixteen splenectomized and 16 intact two-month old white rats were used in this experiment. Base counts were taken on all animals before surgical intervention. The time of ACTH injection of 2 units, per 100 grams of body weight, and the immediate withdrawl of the first blood sample were designated as 0-hour. Subsequent platelet and eosinophil counts were made at one, two, three, four and ten hours post 0-hour.

The splenectomized group was the first to show significant effects of ACTH as reflected by the 33 per cent, 48 per cent and 47 per cent decreases in the platelet means at the one, two and three hour periods, respectively. The splenectomized group went into eosinopenia two hours post 0-hour. The intact group showed a 44 per cent decrease in platelet values at the three hour period and a 41 per cent decrease at the four hour period post 0-hour. The intact group was in complete eosinopenia at the ten hour period. Both splenectomized and intact groups revealed a megakaryocyte increase post-experimentally. The splenectomized animals increased 62 per cent; and the intact animals, 68 per cent.

4. Procedure and results of the ACTH long-term experiment

Fourteen splenectomized and five intact two-month old white rats were used in this experiment. Base counts were made on all animals before surgical intervention. 0-hour was 8:00 a.m. of the first day of the experiment, at which time 2 units of ACTH, per 100 grams of body weight, were first injected and blood samples for the 0-hour count were immediately withdrawn. Daily injections and daily platelet and eosinophil counts were then made over a period of 14 days. Counts were also run on the sixteenth and nineteenth days to ascertain if any tapering off would occur after termination of ACTH injections on the fourteenth day.

The first day's data of this experiment were consistent with those of the short-term ACTH experiment, namely one of platelet value depression. The platelet means rose to 0-hour level on the second day. On the third and succeeding days platelet values continued to rise, culminating in highs on the ninth and eleventh days of a 52 per cent mean platelet increase for the splenectomized group and a 134 per cent mean increase for the intact group. After ACTH injections were discontinued, the platelet means for both groups on the sixteenth and nineteenth days dropped toward the 0-hour levels. From the second to the fourteenth day, inclusive, both groups were in eosinopenia; counts on the sixteenth and nineteenth days showed eosinophils approaching 0-hour levels. The splenectomized group revealed a 92 per cent megakaryocyte increase post-experimentally, and the intact group showed a 153 per cent increase for the same period of time.

5. Procedure and results of the cortisone short-term experiment

The design of this experiment was similar to that of the short-term ACTH experiment. Fourteen splenectomized and ten intact animals were used. Base counts were run on all animals before surgical intervention. The time of the injection of 2.5 mg. of cortisone acetate, per 100 grams of body weight, and the immediate withdrawl of the first blood sample for platelet and eosinophil counts was designated as 0-hour. Subsequent sampling and counting were done at 15, 30, 60, 120 and 240 minute periods.

In the cortisone short-term experiment the platelet means dropped precipitously at the sixty minute period. The most significant decrease was experienced by the splenectomized group whose mean platelet decrease at sixty minutes post 0-hour was 42 per cent. At the two hour period, however, platelet means of both groups were approximately back to 0-hour levels. At the four hour period both groups were in complete eosinopenia. The splenectomized group showed a 51 per cent megakaryocyte increase over its pre-experimental mean, and the intact group showed a 234 per cent increase over the same period of observation.

6. Procedure and results of the cortisone long-term experiment

The design of this experiment was similar to that of the long-term ACTH experiment. Thirteen splenectomized and 14 intact rats were used in this experiment. Base counts were taken before surgical intervention. 0-hour was 8:00 a.m. the first day of the experiment when the first daily injection of 2.5 mg. of cortisone acetate, per 100 grams of body weight, was given and samples were taken for the platelet and eosinophil 0-hour counts. Ten hours post 0-hour, counts were again made which were recorded as the first day's

values. After the first day, and for 12 days thereafter, the animals were injected each day between 8:00 and 10:00 a.m., and platelet and eosinophil counts were made between 1:00 and 3:00 p.m. the same day. This regularity was maintained throughout the experiment. After 12 days of cortisone injecting and platelet and eosinophil counting, the counting was extended to the eighteenth day to ascertain if the blood values would taper off after the hormone administration had been withdrawn. Post-experimental megakaryocyte counts were then made.

An examination of the data of the long-term cortisone experiment revealed that from the first through the fourteenth day platelet means of both groups rose steadily. On the fourth day of the experiment the platelet mean for the splenectomized group was 70 per cent above the 0-hour mean, and the platelet mean for the intact group was 131 per cent above their 0-hour mean. The platelet means for both groups held this high level with very little variation for the 12 days of the experiment. On the fifth day both groups went into eosinopenia from which they never recovered during the length of the treatment. The animals were still in eosinopenia two days after injections of cortisone acetate had been terminated. This fact indicated that both groups were under considerable simulated adrenocortical stress and were responding almost equally. The megakaryocyte data support the platelet response of both experimental groups. The splenectomized group revealed a post-experimental megakaryocyte increase of 61 per cent; whereas the intact group showed an increase of 118 per cent over their pre-experimental mean, nearly twice the increase of the splenectomized rats.

D. Conclusions

1. A preliminary study of the effects of handling, anesthetizing, and injecting of 1 ml. of physiological saline intra-peritoneally upon the blood platelets and eosinophils of the intact and splenectomized rat led to the following conclusions:

   a. That blood platelets were unaffected by this mild form of stress, whereas the eosinophils were sensitive enough to respond, finally, at the two hour period of the experiment.

2. A study of the effect of intra-peritoneal injections of 0.25 ml. of epinephrine (1:10,000), per 100 grams of body weight, on blood platelet, eosinophil and marrow megakaryocyte values of the intact, sham-splenectomized and splenectomized rat led to the following conclusions:

   a. That both intact and sham-splenectomized groups would respond significantly to epinephrine but that there was no significant difference between the response of the two groups. On the basis of these data, no sham-splenectomized animals were run in the succeeding experiments.

   b. The platelet mean increase of the splenectomized rats was not significant, showing only 21 and 24 per cent increases at the fifteen and thirty minute periods, respectively.

   c. Epinephrine action upon blood platelets was immediate and of short duration upon all three experimental groups.

   d. As a $\pm$ 20 per cent change equilibrated with a S. D. of 68,000 in 40, normal, unoperated rats (Ottis, 1951), then blood platelet increases as seen in the intact and sham-splenectomized

groups under epinephrine show a high degree of significance in platelet response to this adrenal hormone.

e. Intact, sham-splenectomized and splenectomized groups all showed significant decreases in eosinophil values at the thirty and sixty minute periods.

f. Contrary to the information in several modern textbooks of physiology, the spleen is not necessary for moderate platelet increases after epinephrine treatment.

3. A study of the effect of two units of ACTH, per 100 grams of body weight, upon blood platelets, eosinophils and marrow megakaryocytes of the splenectomized and intact rat, on a short-term experimental basis over a period of ten hours, led to the following conclusions:

a. Both splenectomized and intact groups showed significant blood platelet decreases at the two and three hour periods under ACTH treatment.

b. That both animal groups were under the same degree of adrenocortical stress was affirmed by the eosinopenic condition of both groups from the third hour to the end of the ten hours of the experiment.

c. The initial effect of ACTH upon platelet values of the splenectomized and intact rat was one of immediate depression; release from this depression was noted at the tenth hour of the experiment.

d. There was a significant increase, post-experimentally, of the marrow megakaryocytes for both groups. The splenectomized group

increased 62 per cent; and the intact group, 68 per cent over the pre-experimental mean. There was, however, no significant difference between groups.

4. A 20 day study of the effect of two units of ACTH, per 100 grams of body weight, daily, upon the blood platelet, eosinophil and marrow megakaryocyte values of the splenectomized and intact rat led to the following conclusions:

a. The first day's data of this long-term experiment were consistent with the results of the short-term experiment. The initial platelet response was one of depression, followed by sharp increases in platelet values.

b. The intact group showed a very significant increase in platelet values, reaching a top of 134 per cent increase on the eleventh day of the experiment.

c. Splenectomized rats responded to a lesser degree but, nevertheless, showed a 52 per cent increase on the ninth day.

d. There was a significant difference between groups in their platelet response to daily ACTH injections. Nonetheless, both groups were in complete eosinopenia from the second day throughout the fourteenth day of the experiment.

e. ACTH was very effective in bringing about thrombocytosis in the intact animal, but less so in the splenectomized animal.

f. Splenectomy in some way modified the animal's ability to respond to this adrenocortical stimulating hormone.

g. The megakaryocyte increases, post-experimentally, supported the platelet data of the experiment.

5. A short-term study of the effect of 2.5 mg. of cortisone acetate, per 100 grams of body weight, upon platelet, eosinophil and marrow megakaryocyte values of the splenectomized and intact rat led to the following conclusions:

a. The early effect of cortisone acetate upon the platelet numbers of splenectomized and intact animals was one of depression.

b. The effect of cortisone acetate upon platelet numbers matched, to a lesser degree, the effect of ACTH in the short-term ACTH experiment.

c. The cortisone acetate depression was short-lived, however, for the platelet values returned to their 0-hour levels at the two hour period and remained there to the end of the observation time.

d. The splenectomized and intact groups were both under equal simulated adrenocortical stress as is affirmed by their complete eosinopenia at the fourth hour.

6. A 20 day study of the effect of 2.5 mg. of cortisone acetate, per 100 grams of body weight, upon platelet, eosinophil and marrow megakaryocyte values of the splenectomized and intact rat led to the following conclusions:

a. The intact group showed a 131 per cent platelet increase on the fourth day and a 148 per cent increase on the sixth day of the experiment. The splenectomized group never rose above the top 70 per cent increase that was established on the fourth day.

b. Once again it may be concluded that the intact animal responded more significantly to hormone induced stress than did the splenectomized animal.

c. Both animal groups reached eosinopenic levels on the fifth day, from which they never recovered during the period of the experiment.

d. The platelet and eosinophil responses noted in the long-term cortisone experiment were similar to those observed in the long-term ACTH experiment, except that the cortisone injected subjects did not recover from eosinopenia, even after eight days post-treatment.

e. It is to be further concluded that splenectomy modified, in some way, the ability of the splenectomized animals to respond to induced hormonal stress with a percentage increase in platelet numbers of a magnitude similar to that of the intact animal.

f. Splenectomy does not protect the animal from a pituitary-adrenal response. This was affirmed by the eosinopenic condition of these animals in both the ACTH and cortisone experiments.

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